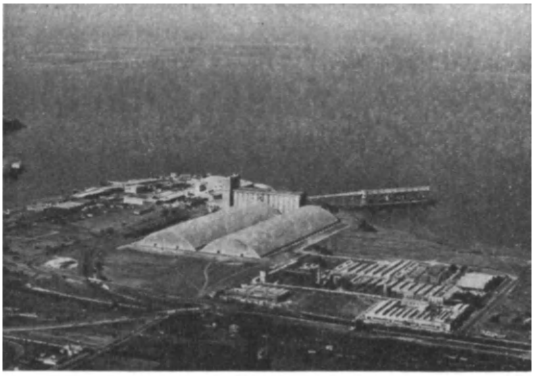
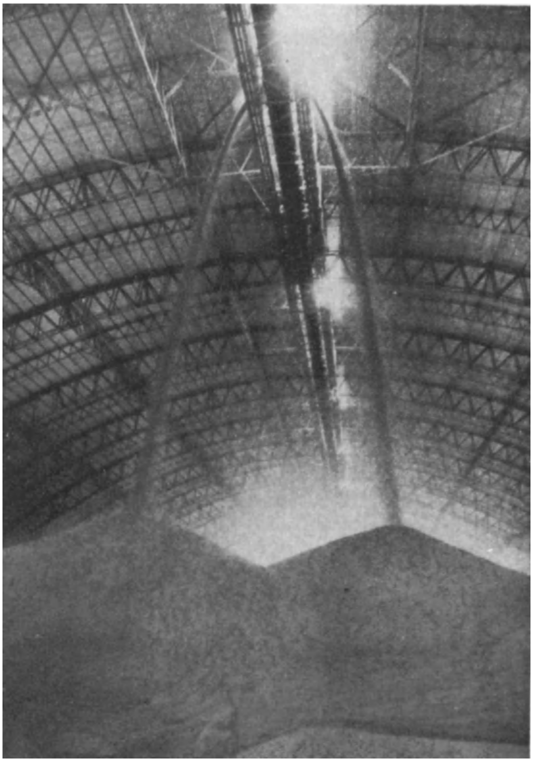


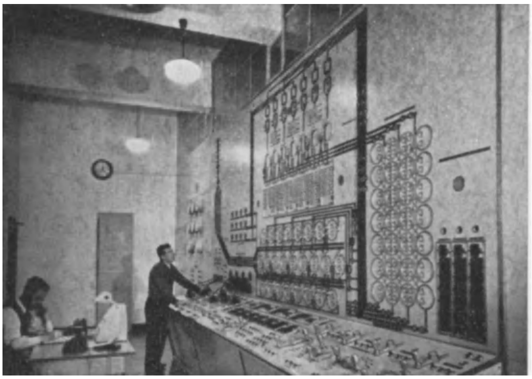
Terminal layout



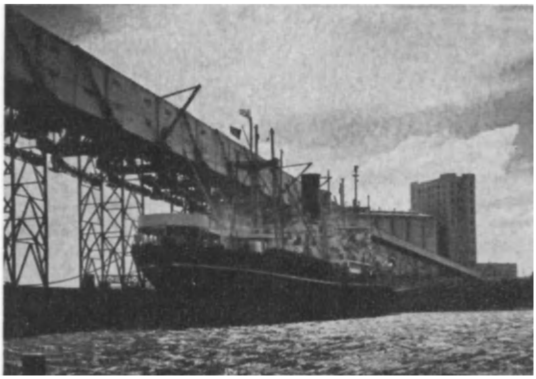
Belt conveyor discharging to storage bin through tripper



Overhead conveyor discharging to horizontal storage



Control room for terminal handling operations



Shipping gallery discharging to boat

Geelong Terminal Elevator (Victoria) Australia

The Entomology of Radiation Disinfestation of Grain

A COLLECTION OF
ORIGINAL RESEARCH PAPERS

EDITED BY

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WITH A FOREWORD BY

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Vienna

PERGAMON PRESS

OXFORD · LONDON · EDINBURGH · NEW YORK

PARIS · FRANKFURT

Pergamon Press Ltd., Headington Hill Hall, Oxford
4 & 5 Fitzroy Square, London W.1
Pergamon Press (Scotland) Ltd., 2 & 3 Teviot Place, Edinburgh 1
Pergamon Press Inc., 44-01 21st Street, Long Island City, New York 11101
Pergamon Press S.A.R.L., 24 rue des Écoles, Paris 5e
Pergamon Press GmbH, Kaiserstrasse 75, Frankfurt-am-Main

First edition 1966

Library of Congress Catalog Card No. 63-23204

1507/65

Dedicated to the

late

LARS-ERIC ERICSON

associated with this work

on behalf of the I.A.E.A.

The work reported in this volume was carried out by the Entomology Group of the Wantage Research Laboratory during the period 1955–61.

Foreword

INSECT damage to stored grains and cereal products has been of great concern to man throughout the ages. The losses incurred have resulted in a waste of his labour and a shortage in his food supply. Various methods of insect control have been devised with varying degrees of success, but there has remained the problem of application at reasonable cost and with no adverse effect upon the product or to the consumer. The increasing use of chemicals has been of concern to the public health authorities because of the possibility of harmful residues.

Irradiation provides us with a new method of preserving a variety of foods and a means of controlling insects in grain and cereal products. Its main recommendations are that it is effective, can be assimilated into modern grain-handling practice, can be used to give instantaneous treatment, and leaves no residue of any type. The application of energy in the form of ionizing radiations is now a recognized addition to the methods of treating food and feeds.

Many of the outstanding contributions which have led to the appreciation of radiation treatment of grain as a recognizably feasible method of insect control are contained in this volume on the Entomology of Radiation Disinfestation of Grain. The International Atomic Energy Agency has been pleased to play a part in the evaluation work that has been carried out into potentialities of the method and in assisting in the development of radiation treatment of grain as an industrial process: within the last year the Agency has sent out three Special Missions to investigate the possibilities of establishing operational pilot plants for radiation disinfestation of grain, to demonstrate the merits and practical economics of this new process. It is my belief that irradiation will soon be established as a recognized method of insect control of stored cereals, and through the Joint FAO/I.A.E.A. Division of Atomic Energy in Agriculture, assistance and guidance in this field will be continued.

It is interesting to note the foundations and teamwork that have gone into irradiation developments. In the middle fifties I thought that research should be started on the use of large radiation sources which were becoming available through the expanded atomic energy programme in the United Kingdom. For this reason the Technological Irradiation Group (as it was then called) at the Wantage Research Laboratory was created as a part of the contribution of the U.K. Atomic Energy Authority to find possible industrial

applications for these new sources. One field of investigation initiated was in radiation entomology and it is of personal satisfaction to me to see that the valuable contributions of this Group have now been reported so well in this book. Work also on the wholesomeness of food treated by ionizing radiation carried out in the United Kingdom and the United States has produced evidence of the safety of irradiated grain for human consumption; formal clearance has already been given in the United States. The Food and Agriculture Organization have now issued a report on the Technical Basis for Legislation on Irradiated Food that should be of great value to many countries in paving the way for international commerce in irradiated food.

This book has been written with an understanding of the needs of the grain-handling industry and presented in such a way as to show how the irradiation process can fit into the handling techniques of that industry with a minimum of disturbance of normal procedures. It is also pointed out that the value of irradiation processing lies in its use as a complement to chemical methods of disinfestation and not as a replacement of them.

Dr. Cornwell and his co-workers are to be congratulated not only for the fine research carried out, but also on the clear and descriptive presentation of their results and guidance.

HENRY SELIGMAN

Editor's Introduction

RADIATION disinfestation of grain is a physical method of insect control, by which grain in motion is treated with a prescribed dose of ionising radiation to inhibit insect reproduction and to kill. It has been the subject of research in a number of countries for the last ten years, and of more recent interest to the International Atomic Energy Agency, Vienna, in fostering practical application of the information now available. The entomological studies reported in this volume were carried out in the United Kingdom in the period 1956–62. The opinions expressed in the Introduction and Discussion to this volume are the personal views of the Editor.

Initiation of research

Early in 1955, a group of specialists of many scientific disciplines was formed within the Isotope Research Division of the Atomic Energy Research Establishment, Harwell, to evaluate the technological uses of ionising radiations in industry and agriculture. A programme of research was initiated into the uses of radiation for the preservation of foods, to include studies on the control of insects in stored foodstuffs, with particular reference to grain. Its objects were to examine the effects of irradiation on insects which infest cereal commodities, on the grain itself in respect of manufacturing properties, taste and possible toxicity, and to evaluate radiation disinfestation as a commercial process in comparison with chemical methods of insect control.

During the early 1950's, interest in the application of ionising radiations for industrial processing was stimulated by the production of waste fission products formed in the uranium fuel elements of nuclear reactors. Prior to that time X-rays were the only source of radiation which could be considered; unsuited to the treatment of bulk materials and costly to produce, disinfestation by X-irradiation, as suggested for instance, as long ago as 1912 in the manufacture of cigars in Florida, never received serious consideration. With the advent of the nuclear power programme and the potential of spent fuel elements and their fission products as large radiation sources, ionising radiation as a means to preserve perishable commodities assumed a new perspective.

The development of nuclear power reactors introduced four possibilities for industrial radiation processing: after removal from reactors, the spent fuel elements themselves could be housed at strategic locations to use

directly the emitted radiation; the fuel elements could be processed to utilise the long-lived gamma-emitting isotopes, such as caesium-137; modifications to reactor design could be made to circulate various products of the nuclear reaction through irradiation facilities alongside; finally the neutron flux of the reactor could be used to produce long-lived gamma-emitting isotopes, such as cobalt-60. So far only the latter procedure has proved of practical interest. The high intensity radiation of spent fuel elements lacks the steadiness required for an industrial irradiation process; only small quantities of caesium-137 have so far been separated from the other fission products, and the placement of irradiation facilities adjacent to reactor plant is of little commercial interest to the food industry. In contrast, many megacuries of cobalt-60 have been obtained by neutron activation; this is the most readily available isotope for industrial processing and can be used to establish irradiation facilities with stable dose rates. It has strong gamma emissions of 1·17 and 1·53 MeV and a half life of 5·3 years. A number of industrial plants utilising the radiation from cobalt-60 are now in commercial operation.

The interest which has developed in the availability of large radiation sources for industrial processing has also stimulated attention to the possible use of electron accelerators. Recent years have shown significant advances in the development of electron machines with considerably greater power outputs than earlier accelerators, but with low beam energy. These also are being used industrially, for initiating various chemical reactions.

In support of the programme of research into radiation disinfestation of stored cereals, two types of radiation source were available for experimental work: a number of cobalt-60 sources, flexible in design and well-suited to the needs of biological research, together with a linear accelerator generating electrons of 4 MeV. The results of the research reported here are confined to the entomological aspects of the process. All but one of the papers are concerned with the effects on insects of gamma radiation from cobalt-60.

Unlike conventional insecticides, which are usually stomach, nerve or respiratory poisons, ionising radiations are non-selective in site of action. Cell nuclei, however, are considerably more sensitive to radiation damage than the cytoplasm, and actively dividing cells, as are present in the reproductive organs, are more susceptible to damage than somatic tissues: radiation may induce molecular re-arrangement at a point on the chromosome and cause gene mutation; chromosome breakage may occur with subsequent rearrangement of gene position or failure to recombine; cell division may be retarded and lead to delayed physiological development. Low doses of radiation may thus cause sterility by induction of dominant lethals in eggs and sperms and may also inhibit the normal cycle of germ cell formation; higher doses cause death of body cells at a rate which depends upon the dose administered.

The irradiation of grain

Among commodities subject to infestation, grain is particularly suited to disinfestation by irradiation: it is handled at centres in sufficient quantities to make the process economic, it is conveyed mechanically, and the flowing properties of the commodity in bulk may be exploited to absorb a high proportion of the radiation and to provide efficient distribution of dose. As man is considerably more susceptible to ionising radiations than insects, radiation processing requires that the source is contained within a concrete shield. The essential differences between the irradiation process for grain and chemical methods of insect control are:

	Irradiation	*Chemical Treatment*
1.	The product is conveyed to the treatment.	The insecticide or fumigant is brought to the product.
2.	Design of the irradiation plant is compatible with 100 per cent control. Homogeneous treatment.	Efficacy is limited by physical factors of distribution and penetration. Failure frequently results from inefficient application.
3.	The process is automatic. No human error or operator prejudice.	Application is subject to human error.
4.	Design completely hazard-proof.	Operators may be subject to toxic materials.
5.	Nothing is added to the product.	Toxic residues may remain.
6.	Treatment is instantaneous.	Delay may be encountered in holding grain under gas or during airing-off.

Grain production, storage and infestation*

World production of cereals fluctuates around 750 million tons per annum: wheat contributes about one-third, maize and rice, individually, about one-quarter and the remainder consists of the coarse grains, barley, oats, rye and sorghum. Between 80–95 per cent of cereals are retained in countries where they are grown: most remain localised on farms and in village stores, and it is only in countries with well-developed storage and elevator systems that cereals are handled in bulk or enter international trade. These are primarily the U.S.A., Canada, Argentina, Australia and France (Fig. 1). About 20 per cent of wheat, 10 per cent of barley and less than 5 per cent of the coarse grains and rice are exported: all rice is handled in bags and most is exported from Burma and Thailand.

At least half of the world's annual yield of 660 million tons of wheat, barley and maize is produced in countries which have bulk storage and handling

* Information compiled with the assistance of Dr. J. A. Freeman, Chief Entomologist, Infestation Control Laboratory, M.A.F.F., London.

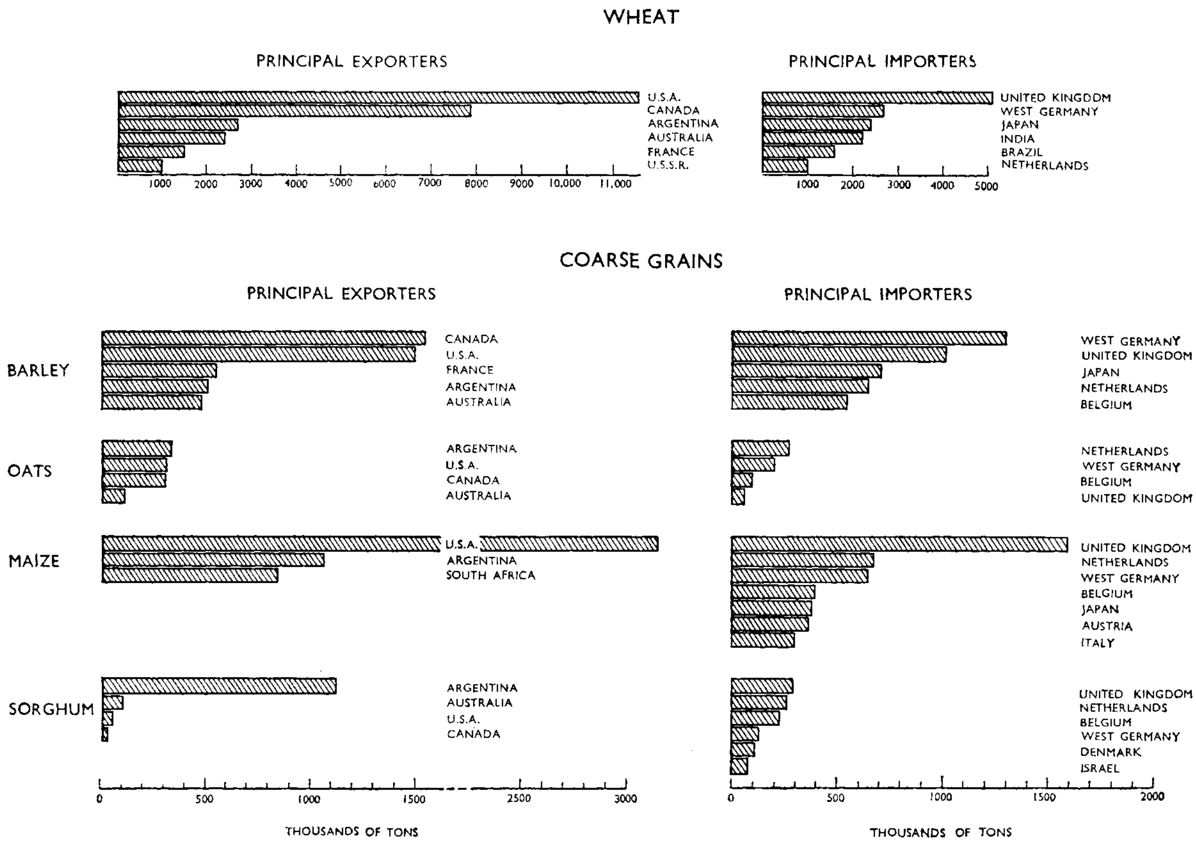


FIG. 1. World exports and imports averaged for 1955-6 to 1958-9

facilities for all or an appreciable part of their stocks. This proportion is increasing as countries undergo economic development and more modern systems of grain handling are installed.

The main losses from storage pests occur in tropical countries where insects breed and spread rapidly; up to 30 per cent of grain is infested seriously enough to consider insecticidal treatment. Low levels of education and extreme poverty make the use of insecticides a difficult problem: here only the most simple control methods are possible except in large stores where adequate technical supervision is available. In the more developed countries a number of techniques are used to prevent and control infestation of grain. These include admixture of insecticides, lowering of temperature by blowing cool air to arrest, or reduce insect development, hermetic storage and fumigation. The admixture of grain with residual insecticides, to maintain quality and prevent losses by infestation, is forbidden in some European countries and is likely to be even more rigorously controlled in the future. Fumigation may be applied to bagged storage under sheets, to bulk grain in bins, and by forced circulatory systems at terminal elevators. Cereals may also be fumigated in barges and lighters or in elevator systems at import. The geographical variation in losses through infestation vary from a few per cent up to 50 per cent according to handling and storage methods.

Grain produced and stored in warm climates must be kept free from infestation if severe loss and damage are to be avoided. Similarly grain imported for storage in such climates must also be free from infestation at time of import. A large proportion of the grain consumed in Britain is imported but the physical loss sustained during short periods in temperate latitudes is considerably less than in the producer countries. Because we have little or no control over infestation overseas, some cereals arrive in Britain infested, either because consignments were contaminated at loading or through resident infestations multiplying in holds *en voyage*. Nevertheless, it is the experience of the Ministry of Agriculture, Fisheries and Food that, with the exception of rice, grain entering British ports, particularly from Canada and the U.S.A., is not seriously contaminated; it is commercially acceptable for immediate processing by measures which eliminate infestation.

Complete freedom from infestation may not be necessary commercially so long as grain is not stored for long periods. Where this is not so, however, it is obviously desirable to exercise effective control, particularly in the tropics where large quantities are held as carry-over stocks, or as national or emergency reserves. In the United States, virtually complete freedom from infestation is required by the Public Health Authorities and in this respect, purity of consignment may be an important consideration for grain entering international trade.

During 1962 a large number of cargoes of wheat (432), barley (107) and maize (350) imported into Britain were examined for infestation; these originated from Canada, the United States, Australia, Argentina, South and

East Africa, France, Rumania, the U.S.S.R. and Iraq. In general, cargoes imported from N. America and from Europe showed little infestation compared with those from countries with warmer climates such as Australia, Argentina and South and East Africa. Table I shows the incidence of the insects which occurred most frequently in these latter cargoes, from which it can be seen that all the well-known beetles are included, together with the moths, *Sitotroga cerealella*, *Cadra cautella*, and *Plodia interpunctella*. Where grain is produced or stored in warm tropical areas, such as Asia, other species may be of greater economic importance, particularly *Trogoderma granarium*.

TABLE I

Incidence of the most common insects in the more heavily infested grains imported into Britain in 1962

Number of shipments examined and per cent infestation

Type of cereal	Wheat	Barley	Maize	Total
Origin of shipment	Australia and South America	Australia	South America and E. and S. Africa	
Inspected	139	61	117	317
Per cent infested	49	41	55	50

Occurrence of the most common insect species per 100 shipments inspected

*Sitophilus oryzae**	**32**	3	**25**	**24**
Sitophilus granarius	4	**23**	1	6
Oryzaephilus surinamensis	**17**	**20**	**14**	**16**
Tribolium castaneum	8	**18**	**24**	**16**
Rhizopertha dominica	**10**	**11**	2	7
Cadra cautella	**13**	5	**36**	**20**
Sitotroga cerealella	5	—	**10**	6
Plodia interpunctella	2	2	**12**	7

* Including *S. zeamais*

[Information provided by the Infestation Control Laboratory, Ministry of Agriculture, Fisheries and Food, based on reports from the Regional Insect Inspectors of that Ministry and of the Department of Agriculture and Fisheries for Scotland.]

Clearly, where there are local conditions which limit the effective application of proven chemical insecticidal techniques, radiation control of insects in stored cereals is also inapplicable. Radiation disinfestation of grain is most

suited to countries with port elevators, equipped with mechanical handling, importing or exporting cereals in bulk; it may be considered as an alternative to fumigation, principally for the treatment of cereals intended for human consumption, notably for wheat, and for barley to which special commercial requirements apply. Irradiation is not of immediate interest for the treatment of cereals used as animal feed (rye, oats, maize, sorghum), in which infestation is of limited significance; neither is it applicable to rice handled in bags, nor to the 90 per cent of cereals which remain up country in the areas of production. It may, however, be considered for the treatment of processed cereal commodities, preferably after packaging.

The programme of entomological research

The contribution of entomological research (Table II) to the application of ionising radiation for disinfestation of grain is to provide the necessary data on which to base proposals for the design of irradiation plant and to assess costs in relation to existing methods of control. Its principal functions are to evaluate the minimum effective dose level for industrial application and to indicate the assurance with which such a figure can be used; to study the factors which influence the radiation susceptibility of insect pests and to indicate what possible factors encountered in practice are likely to reduce the efficacy of the evaluated dose. Research should indicate the outcome of deliberately or accidentally using a dose below the evaluated level, and provide information on the degree of tolerance required for dose uniformity during irradiation. Information is required on the susceptibility of species, developmental stages and strains to compare the merits of toxic chemicals and sterile sperm as insecticidal agents. The research programme outlined in Table II, incorporating these features, was initiated in 1956. Work prior to that year had been largely concerned with the effects of large doses of radiation in producing a rapid kill; the object of the research reported here was to evaluate the conditions for complete sterility, with death ensuing after a short interval – thus minimising the dose necessary for control and the cost of the irradiation process.

Laboratory strains of the grain and rice weevils were examined for susceptibility at all stages of development and a dose level evaluated for their effective control. This work is reported in Paper I. Statistical consideration is also given to the degree of confidence with which this evaluated dose may be used. Papers XI and IX report studies on the lesser grain borer and saw-toothed grain beetle, while Paper VIII examines the susceptibilities of the rust-red and confused flour beetles in comparison with grain weevils. On the basis of this information a comparison is made in the "Editor's Discussion" of differences between species in their tolerance to radiation sterilisation and mortality. The effect of population size is examined in Paper VI, which in-

TABLE II

Research programme on radiation disinfestation of stored products (cereals)

RADIATION SUSCEPTIBILITY OF ALL STAGES OF DEVELOPMENT OF THE GRAIN AND RICE WEEVILS

Evaluation of the minimum sterilising dose against laboratory strains of the principal species, *S. granarius* and *S. zeamais*

FACTORS INFLUENCING RADIATION SUSCEPTIBILITY (PHYSICAL)

Temperature before, during and after irradiation	Dose fractionation (Single *v* multiple passes)	Dose rate	Relative biological efficiency of gammas and accelerated electrons

(CHEMICAL)

	CO_2 and O_2 during irradiation	Cereal rearing media	

REVISION OF CRITICAL DOSE LEVEL FOR STERILISATION

Susceptibility of secondary species	Susceptibility of wild strains	Permanency of sterilisation	Infestation density during immature development

CONFIRMATORY TESTS AT REVISED DOSE LEVEL

Irradiation of massive weevil populations	Nutritional adequacy and toxicity: animal feeding trials	Acceptability: baking quality, taste panel studies, storage trials	Screening of minor insect species for radiation susceptibility

INDUSTRIAL EVALUATION OF RADIATION DISINFESTATION

Assessment of trade demand for radiation disinfestation	Information on storage and handling procedures (annual and conveyor handling rates)	Evaluation of commodities most suited to radiation treatment, e.g. packaged cereals, tobacco, quality products (nuts, spices, chocolate), fabrics and fruit	Pilot plant feasibility studies (engineering)

cludes a test of the evaluated dose against a population of 10,000,000 grain weevils in all stages of development.

The environmental factors examined in the following papers include temperature before, during and after irradiation (Paper III) and temperature

resulting from metabolic heat induced during the larval development of densely crowded populations (Paper V). Chemical factors include the effect of oxygen tension during irradiation which may be influenced by respiration of the insects or grain (Paper VI). The susceptibility of insects bred on cereal media, for which the chemical composition has been determined, is also examined (Paper VII). Among physical factors, dose fractionation is of considerable importance. In practice, irradiation of grain could be carried out in a series of interrupted treatments to accommodate high rates of grain handling at terminal intake; a light treatment given in the first instance followed by the completion of treatment some time later may not produce the effect of a single dose. Thus, Paper IV examines the lethal and sterilising effects of low doses of radiation given at prescribed intervals. Two further aspects considered under physical factors, include dose rate or intensity of radiation, (Paper XIII) and the relative merits of gamma radiation from cobalt-60 and accelerated electrons from machines (Paper XII).

The design of irradiation plant for disinfestation of grain is compatible with 100 per cent control; the dose received is governed by the speed of treatment and the physical properties of the radiation. For practical reasons, however, it is pertinent to examine the effects of underdosing caused by possible variations in conveyor speed or conveyor loading. The desirability of underdosing must also be considered in relation to the economics of the process. With most species of insect examined in these studies, tests for susceptibility were made over a range of experimental doses; these indicate the degree of control likely to be achieved below the minimum effective dose. One study with grain weevils (Paper VI) was specifically carried out to test substerilising doses against large populations, and another (Paper I), the periodicity in fertility obtained with substerilising doses. Studies were also made to evaluate the proportions of substerilised insects which might be tolerated without a reduction in efficiency of the irradiation process. Apart from factors associated with plant design, geographical differences in susceptibility inherent in insects, might also result in underdosing. Two studies were carried out with wild strains collected from the field to compare their susceptibility with laboratory standards (Papers II and X).

Other factors which have been examined during research, include the effects of irradiation on the product; there is a sufficient body of information to indicate that grain can be irradiated with doses at least twenty times greater than that required for insect control without the slightest evidence of induced toxicity. Moreover, doses which begin to have adverse effects on manufacturing properties of the grain are some thirty times higher. These aspects are discussed in more detail later. Limited attempts carried out during this investigation, but not sufficiently extensive to report here, indicate no evidence of resistance in progeny of substerilised insects. One aspect which is reported however, (Paper VI) concerns the degree of protection to grain afforded by sterilised insects against contaminants of the same species.

Acknowledgements

In reporting these studies the authors express their appreciation for collaboration by the Pest Infestation Laboratory, of the Agricultural Research Council and the Infestation Control Division of the Ministry of Agriculture, Fisheries and Food. We are also grateful to staff of the Australian grain handling authorities for technical discussions on methods of grain storage, marketing and export practised in one of the world's largest cereal-producing countries.

The authors wish to express their appreciation of the assistance given with statistical analyses by Mr. B. E. Cooper, Theoretical Physics Division, A.E.R.E., Harwell.

The assistance given by the following four members of the Entomology Group not included in the authorship of these Papers requires special mention – Miss J.A. Morris, Miss D.M. Burson, Miss V.J. Martin and Mr. T. Wond. Thanks are also due to many others of the staff of the Wantage Research Laboratory who contributed in some measure to these investigations. The editor is grateful to Miss R.J. Millet, Information Officer, W.R.L. for seeing this volume through press.

PAPER I

Susceptibility of the Grain and Rice Weevils, *Sitophilus granarius* (L.) and *Sitophilus zeamais* Mots.* to Gamma Radiation

P. B. CORNWELL

An examination is made of the effects of gamma radiation on laboratory strains of the grain and rice weevils, *Sitophilus granarius* (L.) and *Sitophilus zeamais* Mots., when reared at 26°C and 77 per cent relative humidity. Three criteria were used to determine radiation susceptibility: (1) the ability of immature stages to develop and emerge as adults from the grain, (2) survival after emergence and (3) the production of adult progeny. The effects of radiation on the complete life-history of *S.granarius* were examined at 24-hr intervals; each stage of development was subjected to 17 doses ranging from 250 to 20,000 rep. Similar observations on all stages of *S.zeamais* allow a comparison of the susceptibility of the two species. Periodicity in fertility in the grain weevil was examined at substerilising doses; dose levels evaluated for commercial disinfestation were tested against large populations.

INTRODUCTION

Considerable progress has been made during the last 10 years in the design and construction of large gamma irradiation facilities housing isotope sources, particularly cobalt-60,[1–6] or spent fuel elements of nuclear reactors.[7–9] Their development, together with that of electrical machines, which generate high energy electrons,[10,11] has been associated with research into the possible use of ionising radiations for the disinfestation of foods, particularly grain.[12,13] Some of the fundamental and applied problems of using radiation for this purpose have been reviewed.[14]

X-rays have been tested on a variety of insects for their killing and sterilising properties by a number of workers.[15–23] This type of radiation, however, is ill-suited for the treatment of products in bulk and is costly to produce. Penetrating radiation from gamma-emitting sources for the treatment of stored products is now available, and will become more widely so in the near future, at prices comparable with the cost of fumigation.[14,24] Many insect species infesting cereal commodities have been tested for susceptibility to the lethal and sterilising properties of gamma radiation.[25–30] The

* Previously known as *Sitophilus oryzae* (L.) (large strain).

susceptibility of veterinary pests[31–34] and soil insects[35,36] has also been examined. Cereal pests in various stages of development have also been tested for susceptibility to treatment with accelerated electrons.[37–39] In addition, there is extensive literature on ***Drosophila*** concerned with the genetic changes caused by irradiation.

In many instances the aim of previous workers using insects of stored products has been to determine the dose required for immediate kill, rather than to induce reproductive sterilisation; the doses used were often so high as to provide little information on the relative susceptibilities of species and less still on those of developmental stages. With the exception of the studies of Hassett and Jenkins,[25] it is not possible to obtain an approximate estimate of the minimal radiation level for effective commercial disinfestation; none of the reports indicate the degree of control likely to be achieved by substerilising treatment.

The present studies were carried out to examine the effects of gamma radiation on the survival and fertility of two major grain-infesting species, the grain and rice weevils, *S. granarius* and *S. zeamais*. These were treated at all developmental stages to evaluate a dose level which could be recommended with confidence for the control of weevils in grain in an industrial irradiation process.

EXPLORATORY EXPERIMENTS

Preliminary experiments indicated that a study of the radiation susceptibility of all developmental stages must involve a dose range from a few hundred rep* to many thousand. Development of immature stages, particularly eggs, is retarded at certain doses (Fig. 1) so that the examination for adults must be delayed until emergence is complete. Death of irradiated adult weevils is delayed for about 2 weeks after treatment so that examination for survival must also be delayed until the survival rate becomes stable; 3 weeks is sufficient for *S. zeamais* and 4 weeks for *S. granarius*. Since only small differences were found with exploratory samples in the survival of males and females, unsexed samples were used: a more detailed study of the susceptibility of the sexes is reported in Paper VI.

EXPERIMENTAL†

A laboratory strain of *S. granarius* and the large strain of *S. zeamais* (*ex* Pest Infestation Laboratory. A.R.C.) were reared and maintained on Manitoba No. 2 wheat at 26 °C and 77 per cent r.h. Samples of grain containing

* This investigation was carried out when the unit of absorbed radiation dose, rep, corresponding to an energy deposition of 97 ergs/g of tissue was widely used in biological studies.

† Full details of experimental technique are given in U.K.A.E.A. Research Group Report AERE R-3065.

newly-laid eggs were incubated to provide immature stages of known age; duration of the developmental stages of both species under the experimental conditions is given in Tables 1–3. Adults of known age were obtained from cultures which were sieved daily.

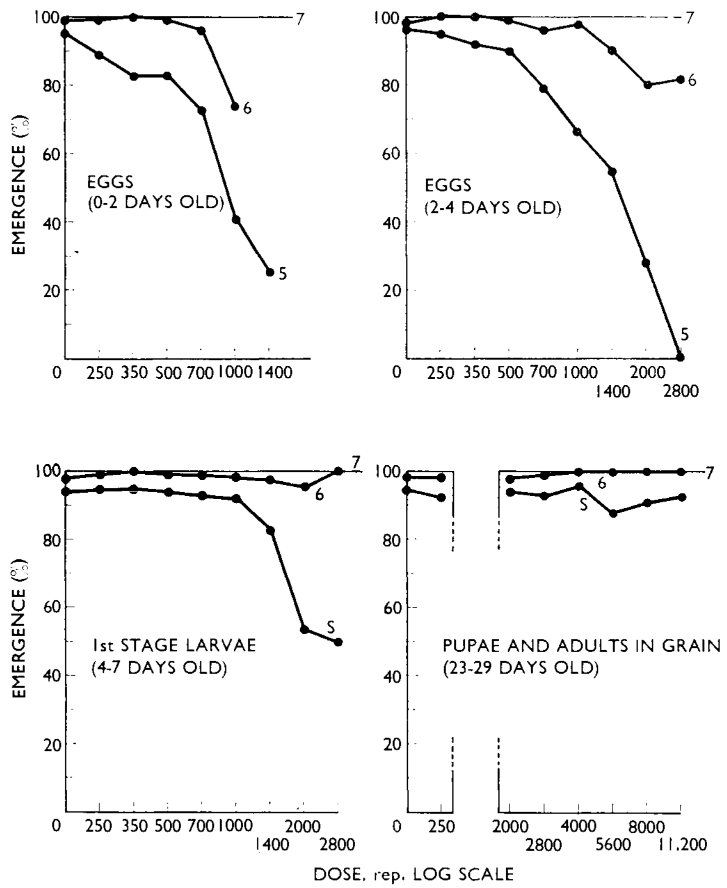


FIG. 1. Effect of age (days after oviposition) on the delayed emergence of adult rice weevils irradiated in the immature stage. Number emerged at 5 and 6 weeks after oviposition expressed as percentage of total at 7 weeks.

The effects of gamma radiation on immature stages of the grain weevil were determined for each 24-hr age interval throughout the life history. Samples of infested grain of about 20 per cent infestation rate were subjected to 17 doses ranging from 250 to 20,000 rep. A minimum of 360 grains and a maximum of 900 were irradiated at each dose, and the controls duplicated. Immature stages of the rice weevil were tested at wider intervals of development, and at doses to a maximum of 11,200 rep. Adult weevils were also

tested for susceptibility at various ages, with particular attention to variations in maturity soon after eclosion. Again, the samples were of a 24-hr age range; two batches of 50 adults were used as a minimum at each dose, and the controls duplicated. The test insects were contained during irradiation in polythene tubes and treated at 25°–35°C in a ^{60}Co source at dose rates from 105 and 135 r/min. Doses were delivered with an accuracy of ±3 per cent. All samples were incubated after irradiation on 100 g of grain. Three criteria were used to determine radiation susceptibility: (1) numbers of adults emerging from the treated grain, (2) survival of these adults after emergence, and (3) production of adult progeny by the survivors.

RESULTS

Emergence

In both species, resistance to radiation increased with stage of development (Fig. 2). Immature stages of *S. zeamais* were more susceptible than those of *S. granarius* (Tables 1–3); only 4 per cent of young eggs of the rice weevil reached maturity when treated with 1400 rep, whereas 17 per cent emerged as adults in the grain weevil, differences in susceptibility being more marked

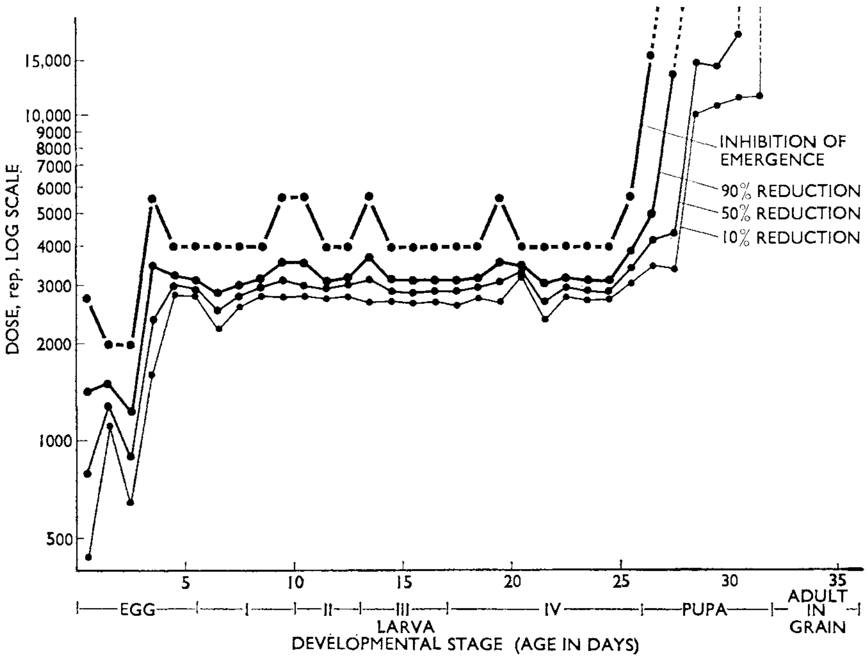


FIG. 2. Increase in resistance of immature stages of *S. granarius* to gamma radiation as shown by changes in the dose inhibiting the emergence of adults and the estimated doses for 90 per cent, 50 per cent and 10 per cent reduction in adult emergence.

at lower doses. More mature eggs of *S. zeamais* treated with 2800 rep gave 3 per cent emergence compared with 72 per cent in *S. granarius*. Irradiation of larval stages of *S. zeamais* with 2800 rep reduced adult emergence to 1 to 6 per cent, a comparable reduction being obtained in *S. granarius* after treatment with 4000 rep. Treatment of pupae of *S. granarius* at this dose level had no effect on adult emergence, but reduced emergence of *S. zeamais* to 78 per cent. Adults in grain of both species were not prevented from emerging by irradiation within the dose ranges investigated.

Survival

Marked differences between the species were also found in the survival of adults which developed from irradiated immature stages (Tables 1–3); whereas 2800 rep was sufficient to kill all adults derived from irradiated eggs

TABLE 1

Emergence (per cent) of adults from irradiated eggs of S. granarius and S. zeamais. Survival of adults (per cent) 4 and 3 weeks, respectively, after emergence in parentheses

Species	*S. granarius*	*S. zeamais*	*S. granarius*	*S. zeamais*
Age (days)	0–3	0–3	3–6	3–5
rep				
700	74 (73)	57 (31)	100 (98)	100 (92)
1000	48 (46)	21 (7)	100 (99)	100 (88)
1400	17 (16)	4 (0·8)	99 (99)	98 (60)
2000	4 (3)	1 (0)	96 (81)	48 (6)
2800	0	0	72 (43)	3 (0)
4000	0	0	0·6 (0)	0

TABLE 2

Emergence (per cent) of adults from irradiated larval stages of S. granarius and S. zeamais. Survival of adults (per cent) 4 and 3 weeks, respectively, after emergence in parentheses

Stage	I		II and III		IV	
Species	*S. granarius*	*S. zeamais*	*S. granarius*	*S. zeamais*	*S. granarius*	*S. zeamais*
Age (days)	6–10	5–7	10–17	9–13	17–26	15–20
rep						
2000	100 (97)	60 (33)	100 (99)	81 (51)	100 (91)	38 (14)
2800	60 (54)	1 (1)	73 (57)	6 (0)	79 (60)	1 (0)
4000	0·3 (0)	0	0·6 (0·4)	0	0·5 (0)	1 (0)
5600	0	0	0	0	0·3 (0)	1 (0)

and larvae of *S. zeamais*, 4000 rep was required to produce the same effect in *S. granarius*. Very small numbers of pupae and adults of the rice weevil survived 5600 rep compared with 12–30 per cent in the grain weevil. For both species, 4000 rep was sufficient to kill almost all adults derived from irradiated eggs and larvae, and 11,200 rep for pupae or adults in grain.

The radiation susceptibility of adult weevils also varied with age (Table 4). With treatment at 4000 rep, samples of *S. granarius*, 2–5 days old, in which the females were fertilised but had not begun to lay, were more resistant (93 per cent survival) than younger or older samples (83 per cent). Adults

TABLE 3

Emergence (per cent) of S. granarius and S. zeamais from irradiated pupae and adults treated in grain. Survival (per cent) of adults 4 and 3 weeks, respectively, after emergence, in parentheses

Stage	Pupae		Adult in grain	
Species	*S. granarius*	*S. zeamais*	*S. granarius*	*S. zeamais*
Age (days)	26–32	22–24	32–36	28–29
rep				
2800	100 (95)	84 (19)	100 (98)	100 (56)
4000	99 (69)	78 (6)	100 (94)	100 (18)
5600	76 (12)	66 (0·6)	100 (30)	100 (0)
11,200	77 (0)	66 (0)	100 (0)	100 (0)

TABLE 4

Percentage survival of adult S. granarius and S. zeamais 28 and 21 days after irradiation, respectively. (Data corrected for mortality in controls)

Species	Physiological age	Age (days)	Dose (rep) 2800	4000	5600
S. granarius	Before fertilisation	0–2	96	83	15
	Fertilised but prior to oviposition	2–5	100	93	21
	During commencement of oviposition	5–10	98	83	14
	During maximum oviposition	10–21	98	78	11
	During declining oviposition	55–56	95	66	9
S. zeamais	Before fertilisation	0–1	86	29	0
	Fertilised but prior to oviposition	2–4	87	33	2
	During commencement of oviposition	5–8	94	62	5
	During maximum oviposition	13–21	97	59	1

aged 0–2 and 5–10 days were of equal susceptibility, but more resistant than those older than 10 days (78 per cent – 66 per cent). Variations in the response of adult rice weevils were more marked; survival was higher at 4000 rep after the commencement of egg-laying (62 per cent) than before (29 per cent and 33 per cent).

The survival curves for adults of both species (Fig. 3) are characterised by delayed mortality after irradiation; this period is about 14 days after the treatment of 4–5-day-old grain weevils, and 9 days for rice weevils 0–1-day-old. The period of delay is unaffected by doses within the range 6000 to 12,000 rep for *S. granarius* and 4000–10,000 rep for *S. zeamais*. It is, however, reduced to 10 days for the grain weevil when adults 8 weeks old are irradiated

Fertility

Changes in susceptibility to sterilisation at 24-hr intervals during development are shown for the grain weevil in Fig. 4. The immature stages of both species were markedly susceptible as young eggs and last-stage larvae. There was a gradual increase in resistance to sterilisation with development from eggs to second stage larvae (Tables 5–6); second and third stage larvae were

TABLE 5

Total progeny of S. granarius and S. zeamais after the irradiation of eggs. Percentage of control in parentheses

Species	*S. granarius*	*S. zeamais*	*S. granarius*	*S. zeamais*
Age (days)	0–3	0–3	3–6	3–5
Control	5740 (100)	10,626 (100)	4878 (100)	5160 (100)
350 rep	4402 (77)	8705 (82)	5097 (105)	5570 (108)
700	3202 (56)	2443 (23)	5067 (104)	4678 (91)
1000	1284 (22)	144 (1·3)	3529 (72)	3214 (62)
1400	557 (10)	0 (0)	2330 (48)	1406 (27)
2000	127 (2·2)	—	1001 (21)	2 (0·04)

Dash signifies no adults emerged to test for fertility.

equally susceptible, but were more resistant than the fourth stage, these being comparable in susceptibility with old eggs. Adults irradiated as pupae and before emergence from the grain (Table 7) were more resistant to sterilisation and showed slight residual fertility at doses above 4000 rep, occasional progeny being produced up to 12,000 rep. Differences in age of adult weevils had only a slight effect in modifying the effects of irradiation on progeny production.

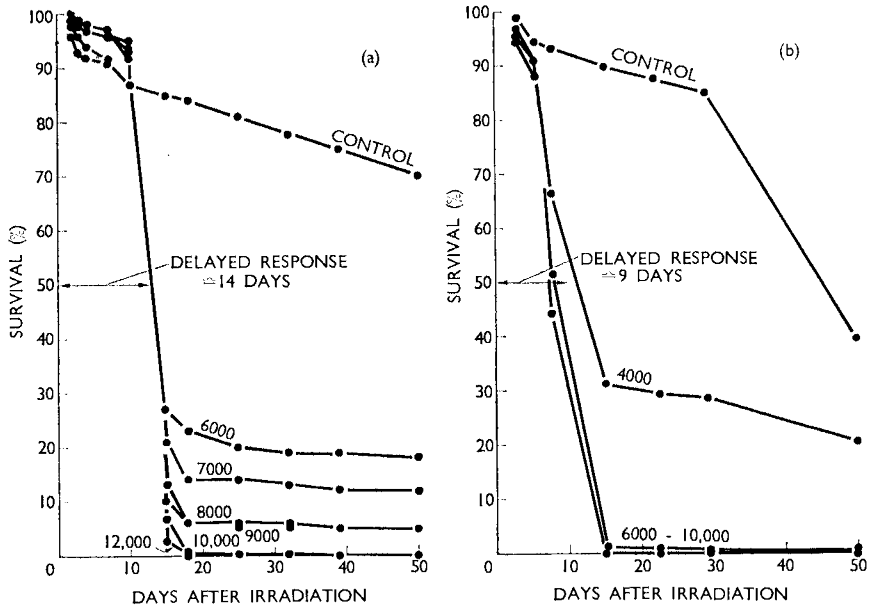


FIG. 3. Time/response curves for (a) survival of adult *S. granarius* (4–5 days old) and (b) *S. zeamais* (0–1 days old) treated with gamma radiation (rep).

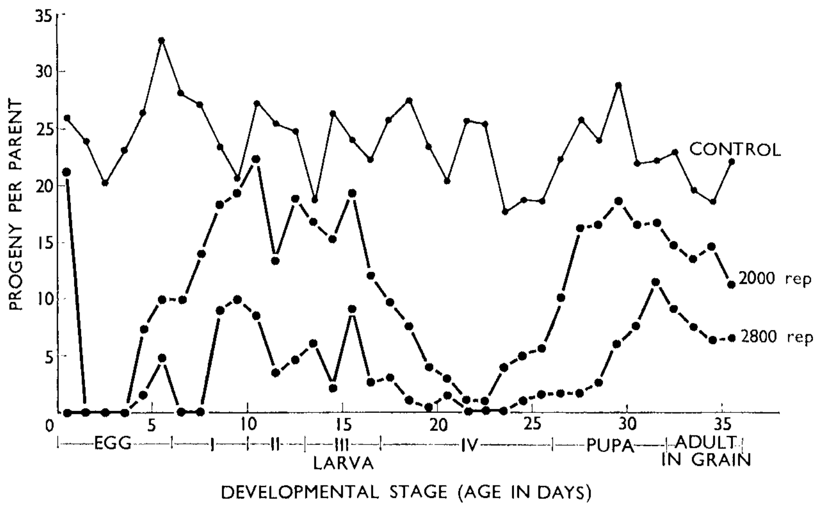


FIG. 4. Effect of age on the fertility of *S. granarius* irradiated in the immature stage.

TABLE 6

Total progeny of S. granarius and S. zeamais after the irradiation of larvae. Percentage of control in parentheses

Stage	I		II and III		IV	
Species	*S. granarius*	*S. zeamais*	*S. granarius*	*S. zeamais*	*S. granarius*	*S. zeamais*
Age (days)	6–10	5–7	10–17	9–13	17–26	15–20
Control	5652 (100)	6050 (100)	14,232 (100)	6300 (100)	14,002 (100)	7315 (100)
2000 rep	3946 (70)	299 (4·9)	9758 (69)	939 (15)	2911 (21)	117 (1·6)
2800	1259 (22)	0 (0)	2299 (16)	—	420 (3)	0 (0)
4000	0 (0)	—	0 (0)	—	0 (0)	0 (0)

Dash signifies no adults emerged to test for fertility.

TABLE 7

Total progeny of S. granarius and S. zeamais after the irradiation of pupae and adults in grain. Percentage of control in parentheses

Stage	Pupae		Adults in grain	
Species	*S. granarius*	*S. zeamais*	*S. granarius*	*S. zeamais*
Age (days)	26–32	22–24	32–36	28–29
Control	10,452 (100)	5782 (100)	7988 (100)	2515 (100)
2000 rep	7570 (72)	548 (9·5)	4488 (56)	352 (14)
2800	2535 (24)	40 (0·7)	2947 (37)	73 (2·9)
4000	238 (2·3)	1 (0·02)	236 (3)	0 (0)

TABLE 8

Total progeny of S. granarius and S. zeamais after the irradiation of adults. Percentage of control in parentheses

Species	*S. granarius*	*S. zeamais*
Control	47,430 (100)	19,332 (100)
2000 rep	21,504 (45)	4798 (25)
2800	9531 (20)	2033 (11)
4000	2940 (6·2)	555 (2·9)
5600	426 (0·90)	100 (0·52)
8000	78 (0·16)	11 (0·057)
11,200	13 (0·027)	7 (0·036)

TABLE 9

Numbers of progeny of S. granarius derived from eggs laid

Number of adults irradiated	Dose (rep)	Oviposition period			
		0–2	2–4	3–7	6–10
550	Control	262	290	1198	1385
650	6000	1	7	74	27
650	6500	1	8	65	17
650	7000	0	1	52	16
650	7500	1	5	38	13
750	8000	1	0	46	13
750	8500	0	1	30	10
750	9000	0	4	12	2
750	9500	0	0	17	3
850	10,000	0	1	9	1
850	12,000	0	0	0	0

Dash signifies total death of parents

The greater susceptibility of all stages of *S. zeamais* to sterilisation (Tables 5–7) is anticipated in view of the greater susceptibility of this species to killing by irradiation. Adults of the rice weevil (Table 8) produced about half the progeny at all doses: the numbers obtained at 11,200 rep (0·03 per cent of controls) were too few for accurate comparison.

Periodicity in fertility of grain weevils after irradiation with substerilising doses was examined by treating adults 0–8 weeks old and transferring them to fresh grain periodically. Irradiation caused an almost complete cessation of progeny production for 2 days after treatment (Table 9), fertility being regained on the third and fourth days. Greater numbers of viable eggs were laid between 3 and 10 days, particularly at the lower doses, followed by a further break in fertility, of varying duration, at about 9–15 days, when most adults died. Slight fertility of the survivors was then regained and maintained until about 60 days.

STATISTICAL EVALUATION OF A DOSE LEVEL FOR COMMERCIAL DISINFESTATION

A dose level evaluated for commercial application of radiation is required with a certain degree of confidence to provide 100 per cent control. Results of the laboratory tests have indicated that the radiation level for inhibiting reproduction of *S. granarius* lies close to but above 11,200 rep. The rice weevil is clearly more susceptible and a dose level evaluated for the control of grain weevils would thus be effective against both species.

at intervals after irradiation of adults (0–8 weeks old)

(days after irradiation)						
9–15	13–20	18–28	25–34	31–62	59–90	81–118
1856	2088	2357	1416	4237	2358	944
0	2	10	8	2	0	0
3	0	7	0	1	0	0
0	3	9	18	16	0	0
0	1	8	2	6	0	0
0	1	2	1	0	0	0
0	11	11	6	8	0	0
0	0	0	—	—	—	—
0	0	0	0	1	—	—
0	0	0	0	0	0	0
0	0	—	—	—	—	—

Emergence of fertile adults of grain and rice weevils is prevented by irradiation of their eggs and larvae with 5600 rep. Pupae and adults of *S. granarius* in grain are not prevented, however, from emerging even at 20,000 rep, and the susceptibility of these stages must therefore be considered together with free-living adults in evaluating a sterilising dose.

Data from the experiments with *S. granarius* (Table 10) were first analysed to examine the relationship between progeny production and dose. To obtain a better estimate of the untreated standard, the similar results obtained for the control and 250 rep treatments were combined, this being normal practice in analyses of this type.[40] Transformation of percentages to normal deviates failed to provide a straight-line relationship with log dose, but this is to be expected, since progeny production is affected by survival; doses below 4000 rep are non-lethal, but higher doses progressively reduce the oviposition period to about two weeks. Thus, a regression analysis was carried out using the data only for the range 4000–20,000 rep, with each point weighted inversely by its variance. The following regression equation was obtained by iteration; (standard probit analysis technique)

$$Y \text{ (normal deviate)} = -4{\cdot}588 \log \text{dose} + 14{\cdot}882$$

The next step in the analysis was to calculate the probability (q^n) of obtaining no progeny when n is the number (1200) which normally develops from 50 parents in the absence of irradiation. Since we require this probability to afford a reliable degree of confidence (c), say 99 per cent, we can equate

$$c = q^n, \quad \text{or} \quad 0{\cdot}99 = q^{1200}$$

Solving for q:

$$\log q = \frac{\bar{1}\cdot 9956352}{1200} = \bar{1}\cdot 9999963$$

Retransforming, $q = 0{\cdot}9999916$.

Referring to the table of the normal probability function[41] we may obtain q in terms of the normal deviate, $-4{\cdot}304$. Substituting this value in the regression equation, $Y = -4{\cdot}588x + 14{\cdot}882$, where $x = \log$ dose,

$$x = \frac{4{\cdot}304 + 14{\cdot}882}{4{\cdot}588} = 4{\cdot}1818, \quad \text{i.e. } 15{,}200 \text{ rep.}$$

Accordingly, in 99 per cent of cases we may expect no progeny to be obtained at 15,200 rep. At a higher confidence level of 99·9 per cent complete sterilisation may be expected at 18,450 rep.

Further development of a weevil population after irradiation requires the production of at least two progeny, one of each sex. On the 1 per cent of occasions when progeny are obtained at 15,200 rep, there is a high probability, calculated at 99·5 per cent, of there being only one. Two or more progeny are thus likely to be obtained on only 1 in 20,000 occasions, and for these to be of different sex, lowers the frequency to 1 in 40,000. Calculations using the derived equation show that two or more progeny (of the same or different sex)

TABLE 10

Production of progeny by pupae and adults of S. granarius in relation with dose

Dose (rep)	Log dose	Total progeny	No. of observations	Mean progeny	Percentage of control	Normal deviate
0	—	135,647	113	1210·5	(100·0)	—
250	—	49,554	40			
350	2·544	45,499	39	1166·6	96·4	+1·80
500	2·699	45,801	41	1117·1	92·3	+1·43
700	2·845	43,685	41	1065·5	88·0	+1·18
1000	3·000	37,968	41	926·0	76·5	+0·72
1400	3·146	33,697	41	821·9	67·9	+0·47
2000	3·301	26,794	42	638·0	52·7	+0·07
2800	3·447	15,180	56	271·1	22·4	−0·76
4000	3·602	3403	57	59·7	4·93	−1·65
5600	3·748	429	45	9·53	0·788	−2·38
8000	3·903	86	44	1·95	0·161	−2·94
11,200	4·049	17	44	0·39	0·032	−3·41
12,000	4·079	2	48	0·04	0·0034	−3·90
13,000	4·114	0	48	0	0	—
14,000	4·146	1	48	0·02	0·0017	−4·15
15,000	4·176	0	46	0	0	—
20,000	4·301	0	46	0	0	—

may be obtained on 1 per cent of occasions at doses below 11,580 rep and on 0·1 per cent of occasions below 13,350 rep.

It must be pointed out that these calculations relate specifically to the experimental conditions described, and include counts of progeny some of which were dead at examination (this was found particularly marked at 11,200 rep) and which were not tested for their ability to produce a further generation. Rice weevils irradiated by Peredel'skii *et al.*[23] (5000 to 8000 r) gave only 25 per cent of progeny in the second generation, and work at this laboratory on grain weevils and *Oryzaephilus surinamensis* (to be described in later papers) indicates that reproductive integrity of progeny is much reduced by the treatment of parents at high doses. It is possible, therefore, that doses lower than those evaluated would give a comparable measure of control. These calculations are also based on tests with a standard number of weevils, confined at a certain density under near-optimum conditions for the species. Environmental conditions in commercial storage vary within wide limits and may affect mating and oviposition considerably.

To test the efficacy of the evaluated doses against large numbers of weevils, two populations of 15,000 adults together with about 10,000 pupae and adults in grain were treated with 15,000 rep $\pm$ 6 per cent at 7×10^4 rep/hr. The samples, each of 17 kg of grain, were retained in bulk; after 6 months one was heavily infested, but the other remained free from infestation for a further 12 months. This result is anticipated with the use of marginal dose levels offering a very small probability of residual fertility.

In a second experiment, two larger populations of 1 million immature stages in grain and 1½ million adults were treated at 16,500 rep and sealed against possible contamination; the range in dose given by 10 ferrous sulphate dosimeters was 3·5 per cent and 1·3 per cent respectively. Experiments in the dose range 15,000–18,500 rep with five smaller populations, containing immature insects in all stages of development, showed that only 6·5 per cent of adults emerge, all within 2 weeks of irradiation. Thus the large population of 1 million immature stages produced about 65,000 adults. Four months after the irradiation of immature stages no live adults were found, and there was no indication of reproduction at 6 months. Four months after the irradiation of adults, samples revealed an estimated population of 1873 weevils, 0·12 per cent of the original population; at 6 months the population had dropped to less than 500 (about 0·03 per cent), indicating that the live adults were survivors, and that reproduction had not occurred.

DISCUSSION

In common with other insect species irradiated during development, the grain and rice weevils show a gradual increase in resistance throughout the life cycle. The greater susceptibility of eggs has been observed with a number of insects,[18] including some which infest stored products.[17,39] The present

work has confirmed the marked changes in susceptibility which occur in the egg during embryological development;[42–45] in *S. zeamais*, for which a great deal is known of embryological events under the temperature and humidity conditions used in the present studies,[46] development to maturity was most readily prevented by irradiation of eggs during the first 3 days, a period of active cell division, differentiation of tissues and development of organs. Sterile adults were most readily obtained by irradiation of eggs 2–3 days old during division and migration of the presumptive reproductive cells.

Using emergence of adults and their survival as criteria of larval susceptibility, the present work failed to show marked differences between larval stages; the slight variations from day to day could not be correlated with moulting, possibly because the samples were composed of individuals with too wide a range of physiological age. Using fertility of the emerged adult as a measure of susceptibility, larvae irradiated in the middle of the fourth instar – during division of the primordial germ cells – were, however, particularly susceptible to sterilisation.

Prolongation of development,[47,48] described by Whiting[49] as resembling a radiation-induced diapause, is an expected symptom of radiation damage if treatment prevents the initiation of events leading to moulting or pupation. Since the larval stage of weevils is spent entirely within grains, no observations were made on their longevity. Other workers, using free-living larvae,[17,39] report reduced mobility and feeding with continued survival well beyond their normal developmental period. Even if pupation is initiated, radiation damage received during the larval stage prevents the normal proliferation of imaginal tissues. Among workers reporting delayed pupation of irradiated *Drosophila* larvae[50,51] Bourgin and colleagues[52] attribute the delay to damage to some target, most likely the ring gland, in the anterior third of the body. Delay in development and the inability of pupae to emerge as adults has been attributed by Japanese workers[53,54] to a functional disturbance of the cephalic complex – the source of moulting hormone.

Previous studies with Dipterous pupae of different age[55–57] have revealed a sudden and dramatic change in susceptibility during the pupal period; Mavor,[58] working with *Drosophila*, suggests that this threshold coincides with the completion of mitotic proliferation of the imaginal tissues. The marked change in susceptibility in the present work occurred for the grain weevil 28 days after incubation, very close to the transition from larva to pupa at 26 days. An additional contribution to the appearance of a threshold age in susceptibility could be the longer period of development for young pupae, compared with older ones, between irradiation and emergence; with a longer period for radiation damage to reduce vigour or culminate in death before eclosion, young pupae may well show a seemingly greater susceptibility.

With the use of radiation for disinfestation, fast-killing doses are recog-

nised as being desirable to stop further damage to the grain.[25] Immediate kill of adult *S. granarius* treated with accelerated electrons is obtained at half a million rep and about 90 per cent die 1 week after treatment with 100,000 rep.[37] Similar results are given by Sumarokov[30] using gamma radiation from cobalt-60. With minimal doses for complete mortality and sterility, death of adult weevils occurs within about 2 weeks of irradiation, this period of delayed response being shorter for the rice weevil than for the grain weevil and in the latter species varying with age. Pendlebury[64] has shown that the period of survival after irradiation is considerably modified by temperature, indicating a dependence on metabolic activity.

Studies to examine the periodicity in fertility of grain weevils treated with substerilising doses showed two breaks in the production of viable eggs. These interruptions are probably caused by a number of factors, principally the extent to which irradiation affects egg-laying and mating, and the destruction of gametes in various stages of differentiation. The first break in progeny production may be caused by an immediate disturbance of egg-laying behaviour. The second break may be a delayed effect of irradiation in its destruction of gametes at an early stage of differentiation, so that no fully fertile eggs can be laid. Between these periods, ripening eggs may be more resistant to radiation and capable of embryonic development. The final phase of fertility follows the delayed production of viable gametes some 20 days after irradiation and probably after remating. Similar experiments by La Chance[59] with parthenogenetic females of *Habrobracon* showed only one break in egg-laying, 6–7 days after irradiation, oviposition being unaffected in the first 2 days. This worker states that two stages in the production of eggs are highly radiosensitive; metaphase eggs at the proximal ends of the ovarioles (those ready to be laid) and actively dividing eggs undergoing differentiation. This condition in the weevil would provide a simple explanation of the present results.

One of the primary functions of this investigation was a comparison of susceptibility of the two species. Previous irradiation studies involving grain and rice weevils have revealed the greater susceptibility of *S. zeamais* to killing by irradiation.[26] The studies of Peredel'skii *et al.*[23] with *S. zeamais* and Mortreuil[22] with *S. granarius*, both using X-rays and with similar conditions of incubation (22–24 °C and 70 per cent r.h.), also show the rice weevil more susceptible to radiation sterilisation. These observations are now confirmed. The difference in susceptibility may be partly associated with the more rapid development of the rice weevil than the grain weevil with a consequently higher metabolic status. It is of interest, however, that studies involving chemical treatment show no discernible differences in susceptibility between the two species when fumigated with hydrogen phosphide (Phostoxin)[60,61] or when in contact with malathion used as a grain protectant.[62–63]

The dose required to produce sterility in nine insect species, three of which infest grain (*S. zeamais*, *Rhizopertha dominica* and *Tribolium confusum*) was

tentatively ascertained by Hassett and Jenkins[25] at about 16,000 r*. From investigations with the rice weevil treated with X-rays, Peredel'skii *et al.*[23] conclude that 5000 r is close to the sterilising dose and this value should be used as a basis for developing irradiators for State granary conveyors. Bibergal *et al.*[3] and Topchiev and colleagues[5] quote 10,000 r as sufficient to kill and completely sterilise weevils, and Brownell,[2] acknowledging the efficacy of 10,000 rep in sterilising eggs of the flour beetle and grain weevil, suggests that 25,000 rep be recommended to provide a margin of assurance. The present results have shown that 16,500 rep (16,000 rads) gives complete control of grain weevils in large populations, and the use of lower doses is unlikely to be 100 per cent effective. This dose level may, however, be more than adequate for effective control of large populations of *S. zeamais.* The adequacy of 16,500 rep (16,000 rads) for the control of wild strains of weevil and under conditions of bulk storage, is the subject of further investigations.

CONCLUSIONS

1. The rice weevil is more susceptible than the grain weevil in all developmental stages to the killing and sterilising effects of gamma radiation.
2. Irradiated eggs and larvae of *S. granarius* are incapable of development to adults after treatment with 5600 rep; those of *S. zeamais* fail to develop after treatment with 4000 rep. Pupae and pre-emerged adults of *S. granarius* are not prevented from developing even at 20,000 rep.
3. Complete mortality of *S. granarius* within 28 days of emergence is obtained when stages in grain are treated with 11,200 rep; the corresponding dose level for *S. zeamais* within 21 days of emergence is 8000 rep. The survival curve for adult weevils is characterised by delayed mortality after irradiation; this period is shorter for the rice weevil than for the grain weevil and in the latter species varies with age.
4. Immature stages are markedly susceptible to sterilisation as eggs 1–3 days old in both species, and as fourth stage larvae, 21–24 days old in *S. granarius*, and 15–17 days old in *S. zeamais*. A dose of 11,200 rep is incompletely sterilising to adults of both species, allowing progeny production, compared with controls, of 0·03 per cent. Substerilising doses result in two breaks in adult fertility, 0–2 days and 9–15 days after irradiation.
5. A statistical evaluation of the dose level for commercial treatment shows that with confidence levels of 99 per cent and 99·9 per cent no progeny are expected from 50 grain weevils treated at 15,200 rep and 18,450 rep, respectively, when retained under the experimental conditions described. Tests with large populations indicate that 15,000 rep is marginal for effective control, allowing some residual fertility, whilst 16,500 rep is completely sterilising. Accordingly, 16,500 rep (16,000 rads) is recommended for control of the grain and rice weevils in industrial applications.

* These workers quoted exposure doses in roentgens.

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PAPER II

Susceptibility of Laboratory and Wild Strains of the Grain Weevil *Sitophilus granarius* (L.) to Gamma Radiation

P. B. CORNWELL

Whilst reduced variability in the gene constitution of laboratory strains makes them useful for preliminary studies on radiation susceptibility, wild strains must also be examined in evaluating radiation levels for commercial application. Tests on five laboratory strains and 30 wild strains of *Sitophilus granarius* (L.) from various parts of the world showed marked differences in susceptibility to killing by irradiation but only slight differences in response to sterilisation. The dose level of 16,500 rep, previously evaluated for the sterilisation of large populations of a laboratory strain, may therefore be recommended for the commercial disinfestation of naturally occurring populations.

INTRODUCTION

Results of a previous investigation[1] into the radiation susceptibility of a laboratory strain of the grain weevil, *S. granarius*, which had been reared in culture for at least 10 years, showed that adults treated with doses ranging from 6000 to 12,000 rep survived 10–14 days after irradiation, the "period of delayed response" varying with age of the insect. Three weeks after irradiation the survival rate remained almost constant, being reduced to less than 5 per cent at doses of 8000 rep and above. Tests for fertility indicated that only occasional offspring were produced at doses above 10,000 rep, and 16,500 rep could be recommended for commercial treatment. Whilst genetical uniformity of laboratory insects may be desirable in preliminary toxicological studies, wild strains must also be examined in evaluating radiation levels for commercial application.

The present studies were carried out to compare the radiation susceptibility of 30 wild strains of *S. granarius* with that of the laboratory strain referred to as the "P.I.L. standard", and with five strains obtained from other laboratories.

EXPERIMENTAL

Twelve wild strains were collected from infested grain in storage and manufacturing premises in the U.K. A further eighteen were taken from boat holds, nine from France, five from Argentina and two each from Iraq and Australia. Samples, usually of about 20 adults, were forwarded to these laboratories by inspectors of the Infestation Control Division, M.A.F.F. Two strains in laboratory culture were obtained from the Cooper Technical Bureau (U.K.) and one each from research establishments in Scotland, U.S.A. and Switzerland. The weevils were then cultured on Manitoba No. 2 wheat for 3–6 months at 26 °C and 76 per cent r.h. to provide sufficient numbers for irradiation. The adults, usually in batches of 100, were treated with gamma radiation from ^{60}Co as described previously.(1) The susceptibility of immature stages was not examined.

Studies were first carried out to examine the duration of the delayed response period (time required for 50 per cent kill). Adults, 1–2 days old, of 13 strains, including P.I.L., were irradiated at 6400 rep and 15,000 rep and survival recorded after 7 days, and at daily intervals between 11 and 18 days. The data were corrected to 100 per cent survival at 7 days using Abbott's formula(2) plotted graphically, and the time required for 50 per cent kill determined by inspection. A second series of tests with adults, 0–14 days old, of 15 strains, was carried out to examine survival 12 days after treatment (towards the end of the delay period), at nine dose levels ranging from 2800 to 11,200 rep. Preliminary experiments had indicated that this interval after irradiation was likely to show the maximum differences between strains. In a final series of tests all strains were examined for mortality 28 days after treatment (after the delay period) and for susceptibility to sterilisation. These last tests were carried out in two stages over a period of about a year. In the first, adults of 14 strains, 0–14 days old, were treated at 11 doses ranging from 2000 to 11,200 rep; with nine of these strains, tests were run concurrently with the P.I.L. standard. In the second tests, adults of 21 strains, 0–7 days old, were treated at eight dose levels ranging from 3250 to 6500 rep, the dose range being reduced to approximate more closely to the expected LD 50. Batches of 100 adults were used at each dose level in the first group of tests and 200 in the second.

RESULTS

Duration of the delayed response period for adults of 13 strains irradiated at 6400 rep ranged from 12·3 to 16·6 days and from 10·9 to 16·3 days when treated at 15,000 rep, the lowest values being obtained for the P.I.L. standard (Fig. 1). Adults of two further strains, from the laboratory of the Cooper Technical Bureau, available for irradiation only at 6400 rep, gave delayed

response periods of 12·3 and 10·7 days respectively, indicating a similar susceptibility to P.I.L. An analysis of regression between the delayed response periods for the two dose levels provided a significant correlation coefficient of +0·93 ($P < 0{\cdot}1$ per cent). The difference in delayed response between the

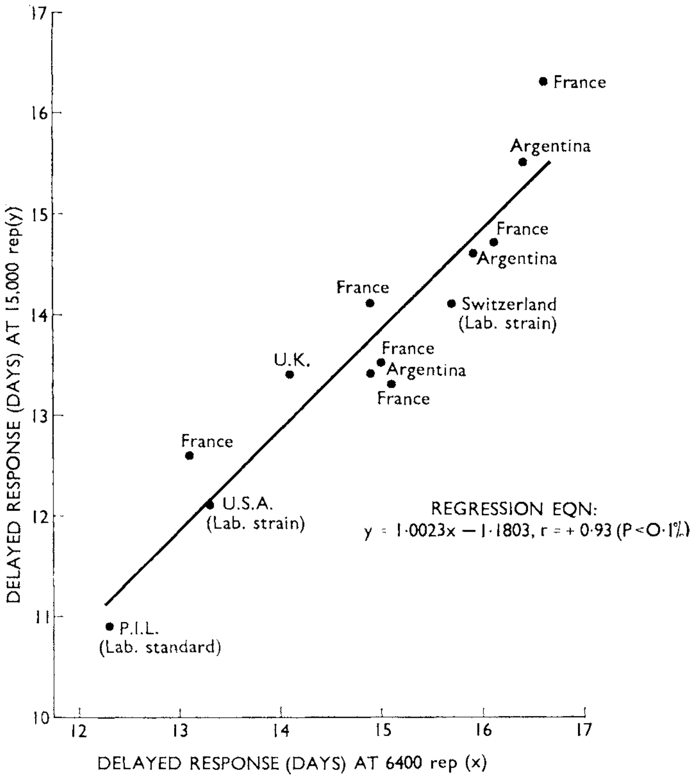


FIG. 1. Relationship between delayed response periods (time required for 50 per cent kill) at 6400 and 15,000 rep for 13 strains of *S.granarius*.

two dose levels was small, about 1 day. The value of near unity for the slope of the regression line indicated that the reduction in time for 50 per cent kill at the higher dose was consistent for all strains tested.

The 15 strains examined in the second series of tests, 12 days after irradiation, summarised for low, intermediate and high dose ranges, showed marked differences in susceptibility (Fig. 2); lowest survival was obtained for the P.I.L. standard (76 per cent, 21 per cent and 11 per cent with increasing dose), and for the two laboratory strains from the Cooper Technical Bureau. The corresponding survival rates for the most resistant strain, from Argentina, were 100 per cent, 94 per cent and 93 per cent.

The data of the third series of tests were treated by probit analysis to derive regression equations relating survival in probits and log dose. Tests on the P.I.L. standard provided a 50 per cent lethal dose of 3950 rep. The fitted lines for P.I.L. and all other strains did not differ significantly in slope, indicating no change in relative susceptibility over the dose range (Fig. 3). Statistical comparison of LD 50 values showed an equal susceptibility of P.I.L. with the two strains* from the Cooper Technical Bureau (3680 and 4050 rep), but all others were more resistant ($P < 0{\cdot}1$ per cent). Two wild strains (from Belgium and France) formed an intermediate group of greater resistance (LD 50s: 4400 and 4610 rep) than P.I.L., but of lower resistance than all others ($P < 0{\cdot}1$ per cent). The laboratory strains from U.S.A. (5160 rep), Switzerland (5220 rep) and Scotland (5500 rep) were comparable in susceptibility to the wild strains; those infesting storage and manufacturing premises gave a range in LD 50 from 5040 to 6780 rep ($P < 0{\cdot}1$ per cent). Those infesting boat holds (with the exception of those mentioned above from Belgium and France) provided a similar range from 5020 to 7060 rep ($P < 0{\cdot}1$ per cent).

Adults of the wild strains, even when reared under the standardised conditions for irradiation, were considerably smaller than those obtained from laboratories, particularly the P.I.L. standard; the average weight of 10 wild strains varied between 1·7 and 2·0 mg, 16 between 2·0 and 2·3 mg and only four were greater than 2·3 mg. All the laboratory strains weighed between 2·3 and 2·9 mg. Using the data for 15 strains, for which very accurate mean weights were obtained, no significant relationship could be established between LD 50 and body weight (correlation coefficient (r) $= -0{\cdot}39$). Attempts to relate susceptibility with country of origin and commodity infested were also unsuccessful: subdivision of all strains with LD 50 values significantly higher than P.I.L. into groups above and below the mean LD 50 (5570 rep) and into categories according to origin (U.K., Europe and New World) showed no marked evidence of heterogeneity ($\chi^2 = 5{\cdot}29$; $P = 10$–5 per cent). Subdivision according to commodity (wheat, barley and other grains) gave a similar result.

Probit analysis was also used to examine the susceptibility of strains to radiation sterilisation; numbers of offspring at each dose level were expressed as percentages of the control, transformed to probits and relationships established with log dose (Fig. 4). Considerable variability was experienced in the numbers of progeny produced by individual strains such that data had to be combined. The strains were grouped as follows: (1) the P.I.L. standard, (2) two wild strains from Belgium and France of intermediate susceptibility to killing by irradiation, (3) 10 wild strains together with those from laboratories of the U.S.A. and Switzerland, each tested in the range 2000–11,200 rep

* One of these was found to be from 3½ to 6½ times as tolerant as the other, of pyrethrins at the LC 50 and LC 80, respectively, and about 60 per cent less susceptible to synergised pyrethrins.[3]

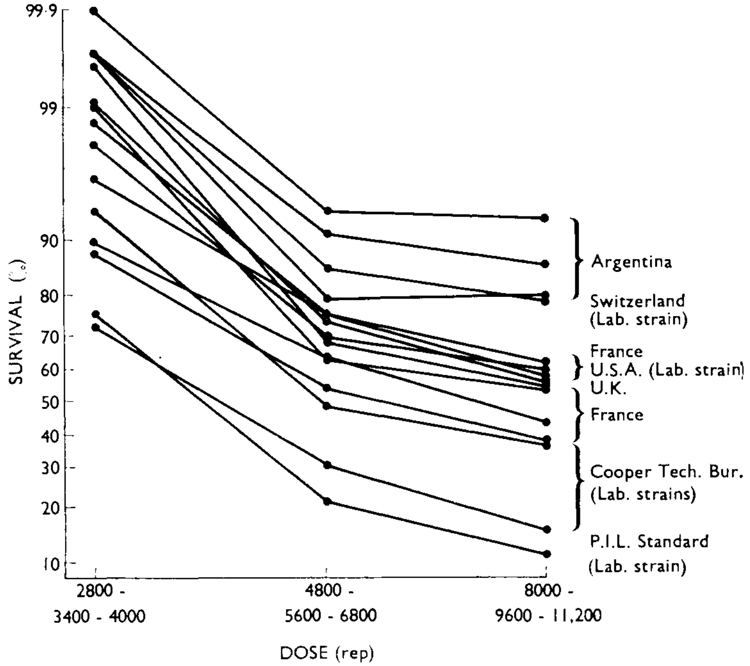


FIG. 2. Percentage survival (Probit Scale) 12 days after irradiation of adults of 15 strains of *S.granarius*. Data corrected for mortality in controls.

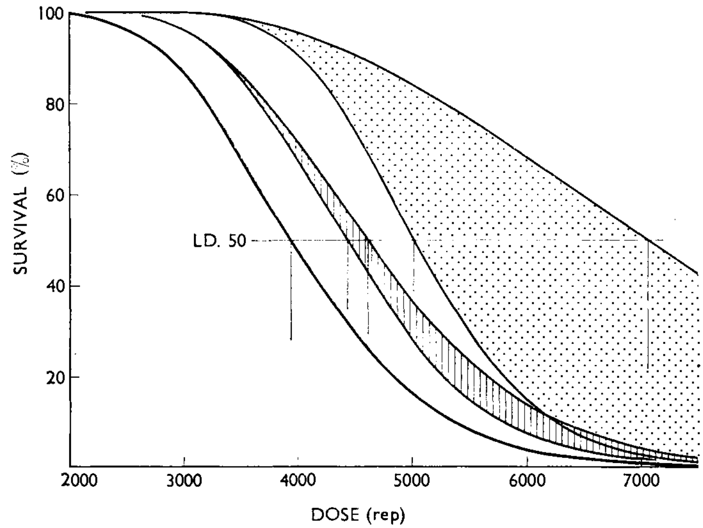


FIG. 3. Fitted dose/survival curves for the standard, P.I.L. strain (thick line), two wild strains of intermediate susceptibility (vertical hatching) and all other wild and laboratory strains excluding those from the Cooper Technical Bureau (range in susceptibility shown by dotted hatching).

with 100 adults at each dose, and (4) 20 wild strains and the Scottish laboratory strain treated between 3250 and 6500 rep with 200 adults at each dose. The fertility of strains from the Cooper Technical Bureau was not examined.

The fitted regression lines were very similar in slope, although significant differences were established between groups 2 and 4 ($P < 1$ per cent) and

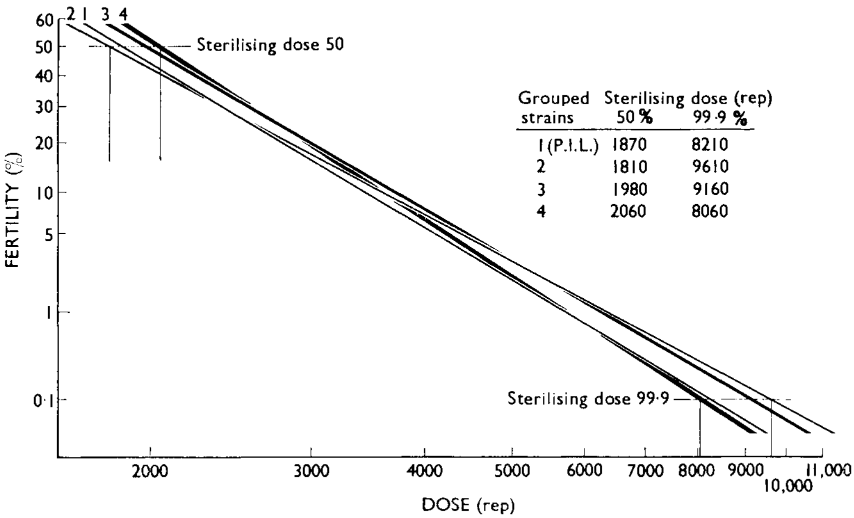


FIG. 4. Fitted dose/fertility relationships for (1) the laboratory standard P.I.L. strain, (2) two strains of intermediate susceptibility to killing by irradiation and (3 and 4) all other laboratory and wild strains (excluding those from the Cooper Technical Bureau).

between groups 3 and 4 ($P < 5$ per cent). There were also only small differences in the dose for 50 per cent reduction in progeny, not statistically significant for P.I.L. and the strains of group 2, or between the strains of groups 3 and 4; the P.I.L. standard, however, was more susceptible than group 3 ($P < 5$ per cent) and group 4 ($P < 1$ per cent). These differences would appear to reflect those previously noted for survival.

Slight differences in the slopes of the regression lines caused the four groups of strains to show a different order of susceptibility at the level for 99·9 per cent reduction in progeny. It must be borne in mind that the variances at the 99·9 per cent level are greater than those at 50 per cent, since the 99·9 per cent level is further from the mean and is thus more affected by errors in the slope of the line than at 50 per cent. Taking into account this extra variability, P.I.L. was equal in susceptibility with other strains, but group 4 was apparently more susceptible than group 3 ($P < 2$ per cent).

DISCUSSION

A number of factors distinguish the P.I.L. standard from wild strains, and from three of the strains obtained from laboratories: the post-irradiation period for 50 per cent kill was shorter for P.I.L.; a greater proportion of individuals in the P.I.L. strain succumbed to irradiation 12 days after treatment than in other strains; the LD 50, 28 days after treatment, was about 1600 rep lower for the P.I.L. standard than for all strains of significantly greater resistance.

The period of delayed response reflects the rate at which radiation damage in the insect culminates in death. Counts of surviving insects 12 days after irradiation provide a further sensitive measure of rate of lethality. The results obtained from both these tests could not, however, be correlated with LD 50, 28 days after irradiation, when rate of lethality had become stabilised. This latter measurement is considered the more reliable expression of strain susceptibility.

Two laboratory strains were similar in all respects to P.I.L.; in the duration of delayed response, numbers surviving at 12 days and LD 50 at 28 days. The range in delayed response period for different strains was similar to that for weevils of different age and for adults of *S. granarius* and *S. zeamais* of similar age.[1] The two laboratory strains known to show considerable differences in tolerance to pyrethrins were comparable in susceptibility to the killing properties of gamma radiation, an expected result with insecticides differing in mode of action. Lack of dependence of the LD 50 on weight of adult, origin, or type of commodity infested suggests that none of these factors alone is primarily responsible for the observed differences in response to killing by irradiation.

Differences in the response of strains to radiation sterilisation may be brought about directly through differences in the susceptibility of the germ cells and indirectly through differences in survival. In the absence of the former, the higher survival rates and consequently increased oviposition periods of the wild strains may be expected to raise their dose level for sterilisation. It has been shown previously,[1] however, that at near sterilising doses the vast majority of progeny were derived from eggs laid 7–11 days after irradiation, before lethality of any of the strains in the present work had become apparent. It seems probable, therefore, that the increased oviposition period plays little part in influencing susceptibility to sterilisation; only differences in the low numbers of adults which survive after the period of delayed mortality can modify the response.

The present results showed that the dose for 50 per cent reduction in fertility of the standard was significantly lower by 110 and 190 rep than for grouped strains with considerably higher survival rates; it was similar to that for two wild strains for which survival rates were only slightly increased. More-

over, near the dose level for complete sterilisation (99·9 per cent reduction in progeny), a disparity in susceptibility between P.I.L. and other strains could not be detected.

CONCLUSIONS

1. Strains of *S. granarius* differ considerably in their rate of response to killing by irradiation – in the post-irradiation period for 50 per cent kill, and in their content of individuals which die within 12 days of irradiation. There is a smaller variation in LD 50, 28 days after irradiation.

2. Differences in the susceptibility of reproductive cells are only slight. The dose level of 16,500 rep (≡ 16,000 rads) previously evaluated for effective sterilisation of large populations of a laboratory strain (P.I.L.) can be applied with confidence for the commercial treatment of cereals supporting naturally occurring infestations.

ACKNOWLEDGEMENTS

I am indebted to the Inspectors of the Infestation Control Division, M.A.F.F., for the collection of wild strains, to the Cooper Technical Bureau, Department of Agriculture for Scotland, Oregon State College, U.S.A., and the Entomologisches Institut, Zürich, who forwarded culture material and to the Pest Infestation Laboratory for supplying the standard.

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PAPER III

The Influence of Temperature upon the Radiation Susceptibility of *Sitophilus granarius* (L.)

J. B. PENDLEBURY

Previous studies on the susceptibility of adult grain weevils to gamma radiation have been carried out at an optimal temperature for the species. The experiment described examines the lethal and sterilising response of adult *Sitophilus granarius* to high and low temperatures, (30° and 15°C) before, during and after irradiation. These are near the limiting temperatures for oviposition and close to the extremes likely to be encountered in commercial practice. Rate of death was considerably increased by high temperature after irradiation. High temperature before irradiation resulted in a slight increase in lethality at doses between 3000 and 10,000 rads when compared with similar batches held at the lower temperature. During irradiation, the influence of temperature was reversed and insects irradiated at 15°C subsequently showed a greater mortality than those irradiated at 30°C. Susceptibility to radiation sterilisation was unchanged. The minimum dose of 16,000 rads evaluated for control of grain weevils would thus be effective at all temperatures likely to be encountered in commercial practice.

INTRODUCTION

The principal application of radiation for control of insects in stored foodstuffs lies in the treatment of bulk grain during terminal handling at export or import. The location of the major grain-producing countries, together with movement of supplies through international trade, results in harvested cereals being subject to a range of temperatures during storage and transit. Variations in temperature might thus occur before, during, or after treatment of the grain, depending upon country of origin and destination, and location of the irradiation plant.

Using large populations of the grain weevil, *(S. granarius)* maintained at 26°C, Cornwell[1] has demonstrated that 16,000 rads is the minimum effective dose of gamma radiation for inhibiting reproduction; this dose also provides 100 per cent kill and is recommended for commercial treatment. Patt[2], however, reviewing research on mechanisms which give

protection to ionising radiation, stresses that temperature has been shown to modify the lethal and sterilising responses of a number of organisms. Very few studies of this type have been carried out with insects and none specifically with those of stored products. The present study was carried out to appraise the efficacy of the dose recommended for commercial application in the light of the lethal and sterilising effects of gamma radiation on the adult grain weevil when temperature is varied before, during and after irradiation.

EXPERIMENTAL

Adult weevils of laboratory strain a obtained from the Pest Infestation Laboratory, were reared on Manitoba No. 2 wheat, standardised by sieving to 30 grains/g. The wheat was fumigated before use with methyl bromide

TABLE 1

Combinations of temperature (°C) before, during and after irradiation

Period		Pre-irradiation	Irradiation	Post-irradiation		
Duration		28 days	4 hr	0–28 days	28–56 days	
Temperature treatment	I	15	15	15	15 30	Ia Ib
	II	30	15	15	15 30	IIa IIb
	III	15	30	15	15 30	IIIa IIIb
	IV	15	15	30	30 15	IVa IVb
	V	15	30	30	30 15	Va Vb
	VI	30	15	30	30 15	VIa VIb
	VII	30	30	15	15 30	VIIa VIIb
	VIII	30	30	30	30 15	VIIIa VIIIb

to eliminate possible contaminants. Stock cultures were obtained by placing 1000 adults to oviposit for 14 days on 1 kg of wheat at 26 °C and 76 per cent r.h. The grain was then incubated for a further 5 weeks and newly emerged adults removed by sieving at daily intervals.

In a factorial experiment (Table 1), weevils 0–1 days old were exposed to the eight possible combinations of two temperatures, (15 ° and 30 °C) before, during and after irradiation. Mortality and sterility were recorded at six dose levels ranging from 3100–20,500 rads, using 100 adults at each dose, and replicated six times. Each irradiated sample was accompanied by a control. All doses were given in an exposure period of 4 hr which resulted in a sevenfold variation in dose rate (775–5122 rads/hr) between the highest and lowest doses. Irradiation temperatures were controlled by placing the sample tubes within constant temperature water jackets.* After irradiation, counts of surviving insects were made at 14, 28, 42 and 56 days, the live adults being transferred to fresh grain on each occasion. To compare the effects of a stable and a varying temperature after irradiation, treatments were split halfway through the post-irradiation period and half the test insects transferred to the higher or lower temperature, as appropriate. Grain on which the insects had been retained was incubated at 26 °C to provide an assessment of fertility.

RESULTS

Mortality

The counts of survivors at intervals after irradiation (Table 2) were examined by analysis of variance. Significant differences were obtained between temperature combinations; rate of mortality was accelerated at 30 °C after irradiation and modified by the temperature before and during irradiation. Rate of death was retarded at 15 °C after irradiation, the response being independent of temperature before and during treatment. Regressions of probit survival at 28, 42 and 56 days after irradiation on log dose were calculated for each treatment; no significant differences were detected in the slopes of the fitted lines and the estimated doses for 50 per cent kill (LD 50) were thus used for comparison (Fig. 1).

Insects maintained at 30 °C before irradiation were significantly more sensitive than those maintained at 15 °C ($P <$ 5 per cent – $<$1 per cent). The LD 50, 28 days after irradiation, could only be calculated for insects maintained at 30 °C *after* irradiation since very few adults died when held at 15 °C. At 42 days, appreciable mortality had occurred in samples held at 15 °C and at 56 days all batches subjected to treatments involving 30 °C, before irradiation showed a greater incidence of mortality than those subjected to 15 °C. The degree of sensitisation varied with dose (Fig. 2); it was greatest at

* Full details of experimental technique and statistical procedures are given in U.K.A.E.A. Research Group Report AERE-R 3641.

7700 rads and entirely absent at the two highest doses, 15,400 and 20,500 rads.

Comparison of LD 50's of samples subjected to treatments identical except for temperature during irradiation (Fig. 1) shows that in all instances, insects

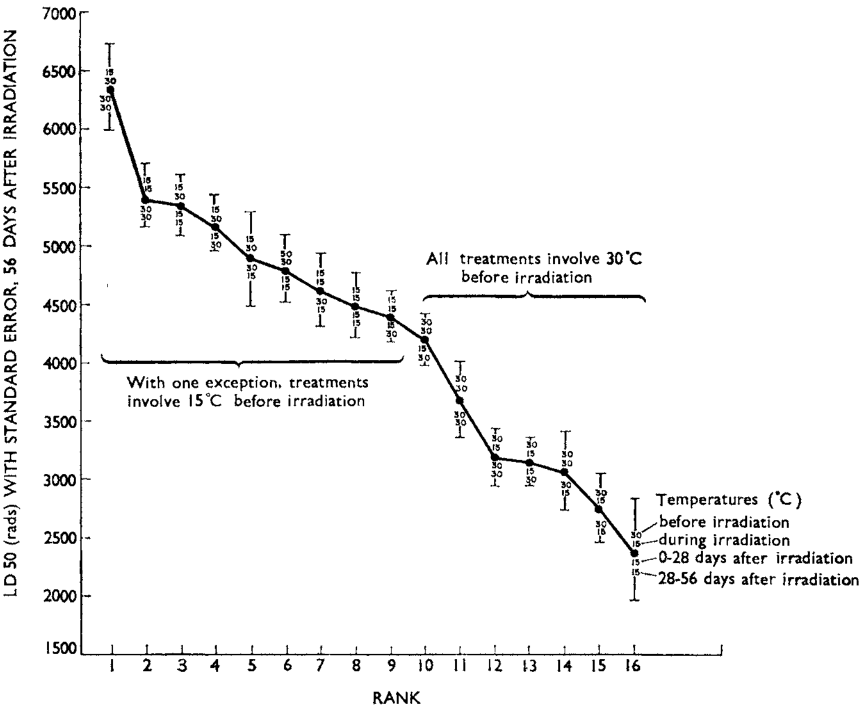


FIG. 1. Ranking of treatments (in order of increasing susceptibility to radiation) at 56 days.

irradiated at 30 °C were more resistant to killing than those irradiated at 15 °C ($P < 5$ per cent–<1 per cent). This effect was again most marked at medium dose levels and not detectable at the upper end of the dose scale. The influence of temperature during irradiation is therefore at variance with its effect before irradiation.

Attention has already been drawn to the considerable influence of temperature after irradiation. The more rapid mortality at high temperature is largely independent of dose and is therefore not reflected in marked changes in LD 50. Inspection of typical survival curves (Fig. 3a) shows that adults retained at 15 °C did not begin to die until 28 days even at 20,500 rads, whereas those maintained at 30 °C were almost all dead by 14 days. In some samples treated with 7700 rads (Fig. 3b), a larger number of adults survived when retained at 30 °C after irradiation, than at 15 °C; these were principally

TABLE 2

Survival (as percentage of control) 14, 28, 42 and 56 days after irradiation

Survival (as % of Control) 14 days after irradiation

Dose (rads)	Temperature treatments I	II	III	IV	V	VI	VII	VIII
3100	103	97	99	102	101	92	100	100
5100	104	99	97	102	98	72	100	97
7700	104	104	101	69	86	1·0	104	42
10,300	100	102	99	3·9	26	0	96	0·4
15,400	92	97	102	0	4·9	0·2	93	0
20,500	100	92	102	0·2	0·2	0	104	0

Survival (as % of Control) 28 days after irradiation

Temperature treatments I	II	III	IV	V	VI	VII	VIII
103	96	99	101	99	90	101	100
103	98	98	98	94	64	100	92
95	102	99	63	81	0·9	101	41
93	100	93	3·2	23	0	92	0·2
95	83	81	0	4·5	0·4	78	0
93	84	89	0	0	0	97	0

Survival (as % of Control) 42 days after irradiation

Dose (rads)	Temperature treatments I		II		III		IV		V		VI		VII		VIII	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
3100	95	89	66	84	104	100	98	102	96	107	84	87	95	100	107	107
5100	83	72	52	31	84	97	107	90	89	100	61	69	75	77	82	97
7700	30	7·1	33	0	42	39	68	60	86	76	0	1·8	33	12	44	54
10,300	28	0	35	0	26	1·8	2·7	3·1	24	23	0	0	37	0	0·7	0
15,400	25	0	42	0	26	0	0	0	2·9	5·8	0·8	0	26	0	0	0
20,500	23	0	21	0	28	0	0	0	0	0	0	0	24	0	0	0

TABLE 2 *(continued)*

Survival (as % of Control) 56 days after irradiation

Dose (rads)	Temperature treatments															
	I		II		III		IV		V		VI		VII		VIII	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
3100	92	90	60	88	102	98	102	101	98	106	78	86	95	97	109	89
5100	56	70	15	23	79	93	97	87	85	98	70	55	58	81	83	94
7700	1·7	7·6	0·4	0	17	40	57	56	86	72	0	0·5	13	6·1	46	34
10,300	22	0	11·9	0	9·2	1·5	3·0	3·2	33	23	0	0	14	0	1·4	0
15,400	0·5	0	2·9	0	0	0	0	0	3·2	5·0	1·4	0	0·2	0	0	0
20,500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

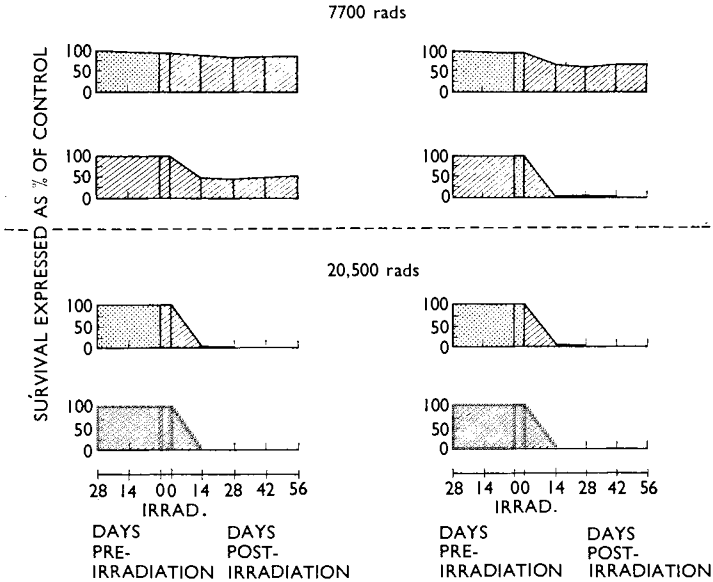


FIG. 2. Influence of pre-irradiation temperature upon survival. Cross-hatched periods indicate insects retained at 30°C, dotted periods at 15°C. An increase of survival with time in some treatments indicates controls dying at a faster rate than irradiated insects.

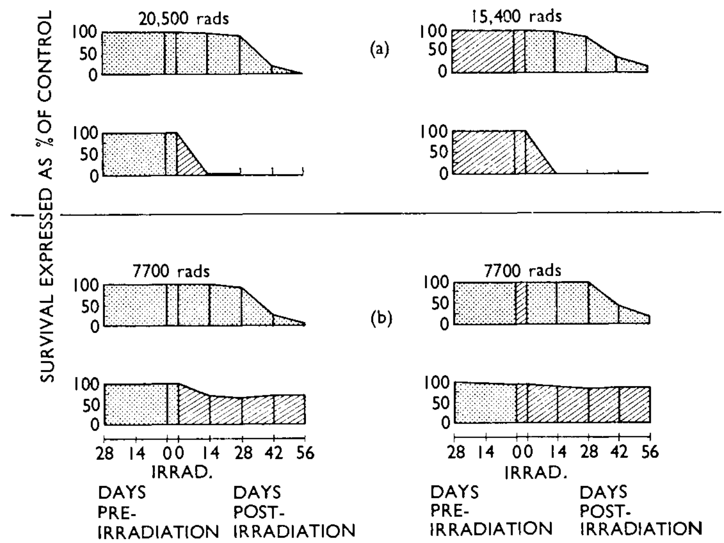


FIG. 3a and b. Influence of post-irradiation temperature upon survival. Cross-hatched periods indicate insects retained at 30°C, dotted periods at 15°C. An increase of survival with time in some treatments indicates controls dying at a faster rate than irradiated insects.

TABLE 3

Progeny totalled for six replicates for 14-day oviposition periods. Progeny as

Dose (rads)	Adult progeny resulting from eggs laid 0–14 days after irradiation: Temperature treatments I	II	III	IV	V	VI	VII	VIII
3100	110 (11)	189 (7·7)	94 (8·2)	2185 (11)	1615 (8·9)	1213 (5·7)	185 (11)	1517 (8·4)
5100	32 (5·0)	53 (3·1)	20 (2·4)	342 (2·1)	346 (1·9)	336 (1·5)	44 (2·7)	356 (2·3)
7700	3 (0·6)	8 (0·5)	4 (0·6)	37 (0·2)	26 (0·2)	19 (0·1)	5 (0·3)	30 (0·1)
10,300	0	0	0	8 (0·04)	3 (0·02)	4 (0·03)	0	3 (0·02)
15,400	0	0	0	6 (0·03)	5 (0·04)	1 (0·01)	1 (0·05)	6 (0·04)
20,500	0	0	0	1 (0·005)	7 (0·04)	1 (0·005)	0	0

Dose (rads)	Adult progeny resulting from eggs laid — Temperature I a	I b	II a	II b	III a	III b	IV a	IV b
3100	80 (17)	788 (18)	24 (6·8)	385 (9·9)	110 (25)	633 (21)	2080 (49)	112 (29)
5100	20 (6·9)	202 (17)	4 (3·2)	24 (1·4)	8 (2·4)	99 (3·0)	62 (1·1)	7 (2·1)
7700	1 (0·2)	6 (0·2)	0	0	0	47 (1·8)	2 (0·06)	0
10,300	0	0	0	1 (0·02)	1 (0·2)	0	0	1 (0·1)
15,400	0	0	0	1 (0·02)	0	2 (0·06)	0	0
20,500	0	0	0	0	1 (0·03)	0	—	—

Dose (rads)	Adult progeny resulting from eggs laid — Temperature I a	I b	II a	II b	III a	III b	IV a	IV b
3100	23 (6·4)	876 (22)	10 (4·6)	89 (3·4)	40 (10)	898 (30)	1108 (47)	89 (32)
5100	7 (2·8)	20 (0·7)	2 (1·4)	8 (0·4)	6 (2)	25 (0·6)	54 (25)	1 (2·6)
7700	3 (1·3)	1 (0·03)	0	—	0	1 (0·02)	0	0
10,300	1 (0·1)	—	0	—	0	0	0	0
15,400	0	—	2 (0·3)	—	0	—	—	—
20,500	0	—	0	—	0	—	—	—

held at 15 °C before irradiation. At doses above 10,300 rads, mortality 56 days after irradiation was similar irrespective of the temperature before, during and after treatment.

Sterility

Treatment with the three lowest doses, 3100, 5100 and 7700 rads, reduced oviposition of fertile eggs during the first 2 weeks after irradiation; an average

percentage of control in parentheses. Dash indicates total death of parents

Adult progeny resulting from eggs laid 14–28 days after irradiation							
Temperature treatments							
I	II	III	IV	V	VI	VII	VIII
165 (41)	96 (14)	157 (47)	5303 (52)	4522 (41)	1395 (18)	121 (17)	1557 (28)
71 (14)	31 (6·7)	26 (9·5)	503 (4·7)	234 (2·5)	163 (6·2)	30 (8·8)	933 (5·8)
4 (1)	7 (1·3)	5 (0·8)	9 (0·07)	10 (0·2)	0	1 (0·1)	282 (11·6)
0	0	1 (0·07)	0	3 (0·2)	0	0	0
0	0	0	—	0	—	0	—
1 (0·1)	0	0	0	0	—	0	—

28–42 days after irradiation							
treatments							
V		VI		VII		VIII	
	b	a	b	a	b	a	b
1465 (46)	121 (33)	294 (11·5)	6 (7·2)	58 (18)	559 (13)	349 (25)	39 (3·5)
45 (1·4)	1 (0·5)	27 (3·1)	1 (0·6)	0	27 (1·0)	473 (19)	3 (7·0)
72 (6·3)	1 (0·3)	0	0	2 (0·3)	46 (2·0)	111 (8·8)	0
0	7 (2·0)	—	—	0	0	0	—
0	0	—	0	0	1 (0·04)	0	0
—	—	—	—	0	1 (0·03)	—	—

42–52 days after irradiation							
treatments							
V		VI		VII		VIII	
a	b	a	b	a	b	a	b
1360 (59)	124 (32)	83 (13)	7 (11)	41 (13)	228 (8·0)	148 (13)	13 (10)
70 (3·7)	2 (1·3)	12 (3·0)	27 (3·9)	1 (0·3)	7 (0·8)	165 (14)	3 (6·8)
0	0	—	0	5 (0·5)	24 (2·7)	44 (18)	0
0	7 (0·7)	—	—	0	—	0	—
—	—	—	—	0	—	—	—
—	—	—	—	0	—	—	—

of about 9 per cent, 3 per cent and 0·3 per cent progeny were produced, respectively, compared with the control (Table 3). The corresponding values for the second 2 weeks rose to 32 per cent, 8 per cent and 2 per cent, respectively, falling to about 20 per cent, 4 per cent and 1 per cent over the following 4 weeks. As very few progeny were produced at the three highest dose levels, analysis of the effect of temperature was carried out on data only for the range 3100–7700 rads. The numbers of progeny obtained in each treatment were expressed as a percentage of the control

and transformed to angles. High and low temperature before, during and after irradiation were found to have no effect on susceptibility to sterilisation.

DISCUSSION

This investigation has shown that quite large modifications in lethality can be produced by varying the temperature before and during irradiation, as reflected in significant changes in LD 50; little modification in lethality is obtained, however, at doses above 10,000 rads. Rate of death is considerably modified by post-irradiation temperature. As LD 50 values at standard times are often used to compare radiation responses of insects, close control of experimental temperatures is at all times of paramount importance.

The literature concerning changes in radiosensitivity due to pre-irradiation heating is relatively scanty, and two of the three available reports concern unicellular organisms. Thus, Langendorff *et al.*[3] show that the LD 50 for the bacterium *Escherichia coli* on nutrient broth can be halved by previous warming to 45 °C. Wood[4] reports that the yeast *Saccharomyces cerevisiae* is sensitised by warming to 52·5 °C prior to irradiation. Baldwin[5], in contrast, shows that the female wasp, *Dahlbominus fuscipennis*, is more resistant to doses of about 250,000 r* when pre-conditioned for 2 hr at 36 °C: at lower doses, neither sex responded measurably to pre-conditioning. It is clear from the ranked LD 50 values in the present work (Fig. 1) that heating before irradiation sensitises adult *Sitophilus* very markedly at doses of 5100 and 7700 rads. The results for the grain weevil do not therefore agree with those of Baldwin for *Dahlbominus*, but the experimental conditions differ considerably, particularly in respect of dose.

Many possible explanations may be given of the effects observed in *Sitophilus*. It has been known for many years[6] that dividing cells are more radiosensitive than resting cells. The increased rate of cell division concurrent with a high level of metabolism could partly explain the increase in sensitivity. Unfortunately, little is known about rates of cell division in adult *Sitophilus*. Jointly responsible with cell division may be physiological ageing: Cornwell[1] has shown that weevils 55–56 days old are more sensitive to radiation than when 2–5 days old. The rate of oviposition and length of the life cycle at 15 °C lead to the conclusion that the physiological age of weevils maintained for 28 days at this temperature is only about a quarter of that at 30 °C. The increase in sensitivity of weevils with chronological (or physiological) age may thus be linked with rate of cell replacement: processes of repair of radiation damage may be less active in the older insects.

Much more information is available concerning the effects of temperature during irradiation. Mottram[7] has shown that the sensitivity of bean root

* The unit of radiation dose, r, (roentgen) is quoted from the original paper.

cells (*Vicia faba*) is increased when irradiated at low temperature and Baker and Sgourakis[8] show an increased frequency of dominant lethal mutations in *Drosophila* when irradiated in the cold; in the absence of oxygen, however, they found no such effect. They conclude that cooling itself is not the primary sensitising factor, but that a higher oxygen tension in cells at low temperature is responsible. Similarly, the radiosensitivity of soaked barley seeds is independent of irradiation temperature in the range 2–20 °C, but is considerably affected by the presence or absence of oxygen.[9] Giles *et al.*[10] have shown that the yield of chromosome aberrations in *Tradescantia* increases as temperature decreases even if the partial pressure of oxygen is kept constant. This effect is attributed to a direct effect of temperature upon recovery processes, particularly the rejoining of chromosome breaks. In *Sitophilus*, one or both the mechanisms outlined above may be responsible for the decreased susceptibility when irradiated at high temperature.

It has already been emphasised that the effects of temperature during irradiation are most pronounced at 5100 and 7700 rads and completely absent at 15,400 and 20,500 rads. Lack of an effect at high doses may be due in some measure to the very small number of survivors; had much larger populations of weevils been irradiated, it might have been possible to demonstrate differences between treatments. The higher dose rates, used to deliver the large doses, may also explain the discrepancy by their not allowing some "recovery" to radiation damage.

In the majority of multicellular organisms, the influence of temperature after irradiation appears to be mainly upon the rate at which damage becomes apparent. Tahmisian and Adamson[11] showed that radiation damage caused by 25,000 r in eggs of the grasshopper, *Melanoplus differentialis*, could be kept latent for 6 months if maintained at submetabolic temperatures. There was no immediate change on returning eggs to 25 °C, but by 8 days the cells had shown pyknosis. Similar delays in lethality at low temperature are reported in higher organisms, notably in amphibia [12,13] and marmots[14]. At 15 °C, onset of death of the grain weevil was delayed until approximately 28 days after irradiation. Complete kill, even with the highest doses, was not achieved until 56 days. This result contrasts markedly with the complete kill obtained within 14 days at 30 °C. Movement of the insects treated with 10,300 rads and above from a low to a high temperature shows that cooling affords no protection against radiation damage and that ultimately (by 56 days) the lethal response at both temperatures is the same (at doses above 7700 rads). At 7700 and 5100 rads, the larger number of survivors at 30 °C after irradiation indicates that processes of recovery to this dose range are aided by a high post-irradiation temperature.

The delay in response at low temperatures may almost certainly be attributed to retardation of metabolism and cell division. Brace[15] postulates that the rate at which a visible expression of radiation damage appears is

dependent upon the rate of cell division. Thus a damaged cell may be capable of existing as a functional unit until, but not after, mitosis. Suppressed synthesis of nucleic acids, the presence of chromosome breaks, cross links and chromosome stickiness may each or all result in abortive cell division. A lowering of metabolism increases the period of mitotic cycle and delays the appearance of radiation effects. Such an effect is reported for radiation damage in chilled rat intestine,[16] where it is believed that delay is due to prevention of the cells from entering mitosis. Other factors unrelated to cell division may also be involved. Much further work is required with insects before the mechanisms responsible can be resolved.

Sterility

Temperature before, during and after irradiation did not affect production of adult progeny. Since the gonads are particularly radiosensitive organs, it is possible that the dose levels (or dose rates) used in these experiments were too high to show differences in fertility. Even so, Jefferies[17] was unable to demonstrate recovery in fertility of adult *S. granarius* subjected to a range of doses, 500–20,000 rep*, delivered with variable numbers of dose fractions and interval times. Further, Bull and Cornwell[18], in experiments on pre-irradiation culture show that environmental temperatures of 26 °C, or 30 °C and above, do not result in differences in numbers of adult progeny. The gonads of weevils are therefore unable to "recover" from large radiation doses or to have their radiosensitivity modified by temperature. That temperature during irradiation at low dose rates may indirectly affect fertility through its influence on fecundity is shown by Grosch[19] who reports that a dose of 11,000 r (dose rate 140 r/hr) at 5 °C is required to prevent egg production in *Habrobracon*. At temperatures above 20 °C, 13,700 r is necessary to produce the same effect.

Efficacy of the dose for commercial disinfestation

It has been shown that at 15,400 and 20,500 rads, mortality and progeny production of *S. granarius* is unchanged by temperatures of 15° or 30 °C before, during and after irradiation. The efficacy of the recommended dose (16,000 rads) in controlling infestations of *Sitophilus* will not therefore be reduced. If a particularly rapid kill is required, some form of post-irradiation heating to raise the grain temperature to 30 °C would be advantageous. If the grain temperature is low, complete kill will be delayed: feeding, however, and hence further damage to the grain has been shown[20,21,22] to be reduced at both low and normal temperatures.

* Rep = roentgen equivalent physical—corresponding to an energy deposition of 97 ergs/g of tissue.

CONCLUSIONS

1. The response of adult grain weevils to killing by gamma irradiation is modified by temperature before and during treatment; rate of mortality is also considerably modified by temperature after irradiation. Susceptibility to sterilisation is unchanged. High temperature before irradiation at 5100 to 7700 rads sensitises weevils to radiation damage, but reduces susceptibility when applied during irradiation. No effect of temperature before or during irradiation is apparent at doses of 10,300 rads and above. High temperature after irradiation accelerates death at all doses investigated; with 15,300 rads and 20,500 rads, complete kill is achieved within 14 days at 30 °C and in 56 days at 15 °C.

2. The mode of action of temperature in modifying the lethal response to gamma radiation probably lies in its effect on cell metabolism, rate of cell division and mechanisms of repair to cell damage. This investigation demonstrates the need for close control of temperature in entomological studies on radiation susceptibility. No loss in the efficacy of the minimum dose (16,000 rads) for control of *S. granarius* is likely to be experienced with the fluctuating and variable temperatures under the commercial conditions in which radiation disinfestation of grain may be applied.

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PAPER IV

Effects of Continuous and Fractionated Doses of Gamma Radiation on the Survival and Fertility of *Sitophilus granarius* (L.)

D. J. JEFFERIES

Megacurie sources of ^{60}Co are required to disinfest grain at 16,500 rep ($\equiv$ 16,000 rads), the dose level evaluated for commercial treatment, at conveyor rates normally encountered in grain handling. Maximum efficiency in the use of irradiation plant might be obtained with sources of lower curie strength to ensure continuous operation if the full dose for sterilisation could be given in a process of repeated passes. The question arises as to whether many sublethal and substerilising doses would provide the same measure of control as continuous-treatment. A study of dose fractionation on the grain weevil shows differences in survival and fertility which may be attributed to "recovery" of somatic and reproductive cells between treatments. Differences in survival were obtained in all developmental stages; recovery was obtained with intervals of 10 min and longer, the process being governed by the number of fractions, the fractional dose, interval time and interval temperature. Recovery in reproductive capacity was obtained in irradiated eggs, larvae and pupae, but not in adults. Whilst these effects are manifest at low doses, fractionated treatment does not adversely affect the degree of control achieved at the commercial dose level.

INTRODUCTION

Radiation disinfestation offers the possibility of instantaneous treatment of grain moving in bulk at normal handling rates in conventional grain storage installations. Calculations have shown that megacurie sources of ^{60}Co are required[1] to treat grain with 16,500 rep ($\equiv$ 16,000 rads), the minimum effective dose for sterilisation of the grain weevil,[2] at 200 tons/hr, commonly used for handling grain at terminal elevators. Smaller sources could, however, be used if treatment were carried out by a series of repeated passes involving the circulation of grain from one storage bin to another. The present studies were carried out to compare the biological efficiency of continuous and fractionated treatments on different stages of the grain weevil, *Sitophilus granarius* (L.); the size of the fractional dose, number of fractions, interval time and interval temperature were also investigated.

EXPERIMENTAL*

A laboratory strain of *S. granarius* was reared and maintained on Manitoba wheat at 26 °C and 76 per cent r.h. Immature stages of required age were obtained by incubating grain on which adults had oviposited for 24 hr; the rate of development of *S. granarius* under these culture conditions has been detailed.[2] Adults of known age were obtained by removing daily the newly emerged insects from stock cultures. Irradiations were carried out using a ^{60}Co source at 5000 rep/hr† and 26 °C, except in the first experiment in which the dose rate varied from 7300 to 10,000 rep/hr and the temperature from 25° to 35 °C. Samples of immature stages consisted of 100 grains of wheat of about 25 per cent infestation rate; doses up to 50,000 rads have been shown not to affect the use of Manitoba wheat as a weevil-rearing medium.[3] Ten samples of eggs, larvae and pupae were used in each treatment; after irradiation, pairs of samples were combined and retained on 200 grains of uninfested wheat. Adults were irradiated in batches of 40 on 100 grains of wheat, with 5 batches in each treatment, and retained after irradiation on 100 g of grain. All samples were held at 26 °C in the intervals between fractionated irradiations unless otherwise stated.

Three criteria were used to assess differences in the radiation susceptibility of developmental stages: (1) number of adults emerging from the grain, (2) survival after emergence, and (3) production of adult progeny. Adults began to emerge from the irradiated immature stages 35–36 days after oviposition and were held collectively on 100 g of grain by periodic transfer. Survivors were removed to fresh grain 28 days after the first transfer and discarded at 56 days. Adults, which survived irradiation, were transferred to fresh grain at 28 and 56 days and discarded at 60 days. An exception was made in the first experiment, in which all stages were irradiated, to examine possible re-development of fertility over a period of 3 months. In all experiments counts of progeny were made 7 and 9 weeks after initial inoculation.

The data for emergence and survival were treated by probit analysis after correcting for mortality in controls.[4] Fertility was assessed by calculating the average number of progeny per live parent for certain periods after the emergence of immature stages and after the irradiation of adults and expressing the results as a percentage of progeny produced in controls. Probit analysis was used to estimate the dose for 50 per cent reduction in fertility; a more accurate estimate of the dose for 99·9 per cent reduction was obtained by treating the relationship with dose which afforded more than a 50 per cent reduction as an exponential function.

* Full details of experimental technique are given in U.K.A.E.A. Research Group Report AERE-R 3503.

† This investigation was carried out when the unit of absorbed radiation dose, rep, corresponding to an energy deposition of 97 ergs/g of tissue, was widely used in biological studies.

TABLE 1

Calculated doses for 50 per cent and 99·9 per cent reduction in emergence, survival and fertility (first month's oviposition) for developmental stages of S. granarius *treated with continuous and fractionated doses of gamma radiation*

	Stage	Treatment	Dose for 50% reduction		Dose for 99·9% reduction	
			log dose ± st. error	Dose (rep)	log dose ± st. error	Dose (rep)
Emergence	Eggs	Continuous	3·102 ± 0·018	1270	3·782 ± 0·052	6060
		Fractionated	3·196 ± 0·042	1570	3·746 ± 0·100	5580
	Larvae	Continuous	3·494 ± 0·004	3120	3·675 ± 0·015	4730
		Fractionated	3·744 ± 0·005	5550	3·911 ± 0·017	8140
Survival	Eggs	Continuous	3·070 ± 0·012	1180	3·631 ± 0·034	4280
		Fractionated	3·197 ± 0·039	1570	3·720 ± 0·101	5240
	Larvae	Continuous	3·481 ± 0·006	3030	3·638 ± 0·014	4350
		Fractionated	3·723 ± 0·007	5280	3·914 ± 0·029	8210
	Pupae	Continuous	3·687 ± 0·011	4860	3·945 ± 0·039	8810
		Fractionated	3·955 ± 0·025	9020	4·153 ± 0·094	14,210
	Adults	Continuous	3·643 ± 0·006	4390	3·964 ± 0·021	9200
		Fractionated	3·814 ± 0·011	6510	4·220 ± 0·043	16,610
Fertility	Eggs	Continuous	3·034 ± 0·033	1080	2350 ± 360 rep	
		Fractionated	3·207 ± 0·043	1610	2890 ± 1330 rep	
	Larvae	Continuous	3·372 ± 0·004	2350	4210 ± 640 rep	
		Fractionated	3·632 ± 0·004	4290	8050 ± 260 rep	
	Pupae	Continuous	3·183 ± 0·069	1520	4700 ± 390 rep	
		Fractionated	3·447 ± 0·017	2800	7150 ± 780 rep	
	Adults	Continuous	3·149 ± 0·028	1410	8680 ± 390 rep	
		Fractionated	3·124 ± 0·050	1330	8800 ± 690 rep	

RESULTS

The effect of four fractions at intervals of 24 hr

In the first experiment continuous and fractionated treatments were given at a range of dose levels to eggs (0–4 days old), larvae (7–11), pupae (28–32) and adults (7–11 days old). Samples given interrupted treatment received the first fractional dose on the first day of the age range; those given continuous treatment contained equal proportions of aged insects spanning the range. Similarly, untreated controls were of two types comparable with the age composition of the two types of irradiated samples.

Regression equations permit calculation of the dose levels which allow 50 per cent emergence (Table 1). With eggs there was no significant difference between continuous (1270 rep) and fractionated treatments (1570 rep);

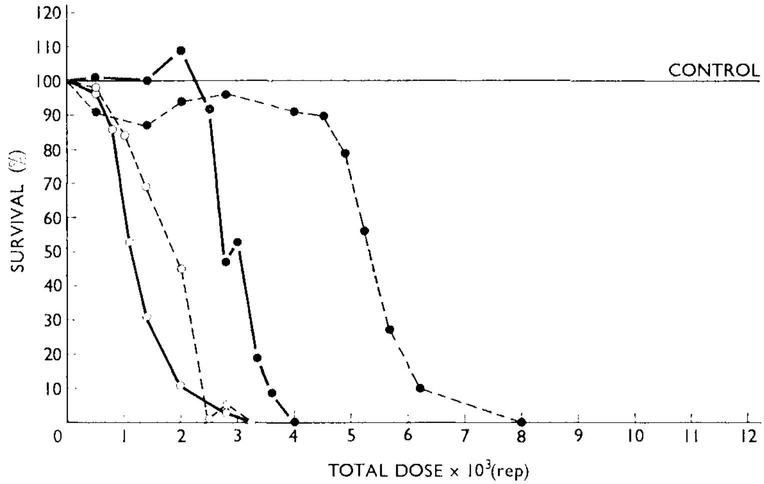


FIG. 1. Percentage survival 60 days after irradiation of eggs and larvae by continuous and fractionated treatments. Survival with fractionated treatments is shown by broken lines.

O – Eggs
● – Larvae

the dose level allowing 50 per cent emergence from irradiated larvae increased from 3120 to 5550 rep ($P < 0{\cdot}1$ per cent). The dose level for 50 per cent emergence from irradiated pupae was outside the dose range investigated.

Further regression equations relating dose and survival of the emerged adults 60 days after irradiation showed that the dose level for 50 per cent mortality (Table 1) was also significantly increased by fractionation (eggs,

TABLE 2

Calculated doses (in rep) for 50 per cent reduction in fertility during the second and third months' oviposition

Stage irradiated	Month of oviposition	Continuous treatment	Fractionated treatment
Eggs	2nd	1250	1690
	3rd	1210	1240
Larvae	2nd	2410	4390
	3rd	2340	4210
Pupae	2nd	1650	2790
	3rd	1350	2560
Adults	2nd	1490	1340
	3rd	1100	1370

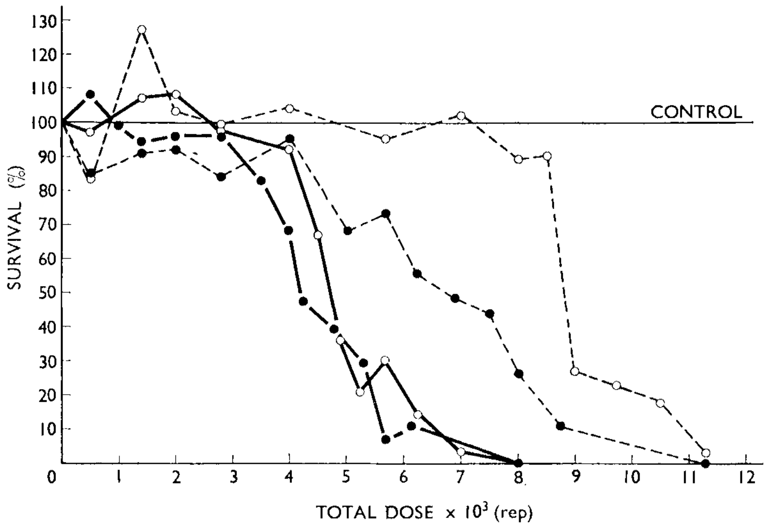


FIG. 2. Percentage survival 60 days after irradiation of pupae and adults by continuous and fractionated treatments. Survival with fractionated treatments is shown by broken lines.

○ – Pupae
● – Adults

P < 1 per cent; larvae, pupae and adults, P < 0·1 per cent), particularly for larvae and pupae (Figs. 1 and 2). The percentage increase over continuous treatment was 34, 74, 85 and 48 per cent for eggs, larvae, pupae and adults, respectively.

The calculated doses for 50 per cent reduction in fertility for the first month of oviposition were significantly increased by fractionated treatment for eggs (49 per cent; P < 2 per cent), larvae (82 per cent; P < 0·1 per cent)

and pupae (84 per cent; $P < 1$ per cent), but the SD 50's and slopes of the fitted regression lines for adults did not differ for the two types of treatment (Table 1). The calculated doses for 50 per cent reduction in fertility during the second and third months' oviposition (Table 2) were similar, in all stages, to those of the first month and were again not increased by dose fractionation in adults. (The 24 per cent increase shown for adults for the third month's oviposition was not significant.) There is, therefore, no foundation in the suggestion from preliminary work[5] that fractionated treatments may lead to the re-development of their fertility. Fewer progeny were produced as the insects aged, complete sterility being reached earlier in the irradiated insects than the controls; those receiving high doses became sterile after 1 month's oviposition. It was not possible, therefore, to calculate dose/fertility relationships beyond the third month.

The doses for 99·9 per cent reduction (Table 1) show that adults require the highest dose to kill and sterilise with both continuous and fractionated treatments; they are therefore potentially the most dangerous stage in the event of inefficient control.

The effect of varying the interval time

Differences in the response of weevils subject to continuous and interrupted treatments indicate the existence of a "recovery" process during the intervals between fractional doses. The second experiment was carried out to examine the dependence of this process on the interval time. To make this

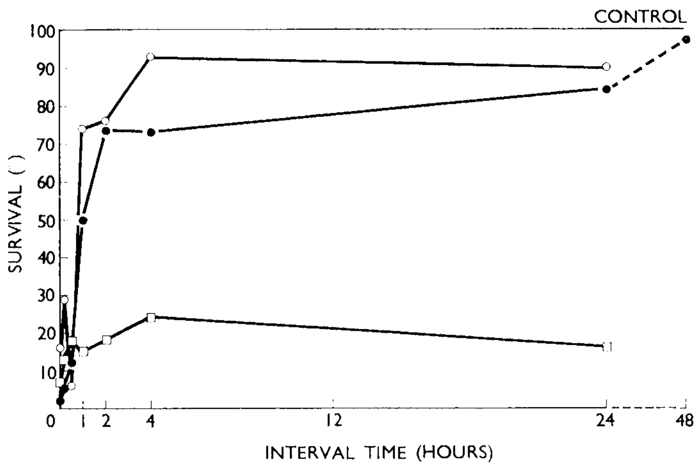


FIG. 3. Survival of developmental stages in relation to interval time in fractionated treatments.

□ – Eggs (4 × 585 rep)
○ – Larvae (4 × 918 rep)
● – Adults (4 × 1627 rep)

study as critical as possible, particularly for the purpose of investigating the effects of short intervals, doses were chosen which afforded 95 per cent mortality with continuous treatment. These were, respectively, 2339 rep (4 × 585) for eggs, 3673 rep (4 × 918) for larvae and 6510 rep (4 × 1627) for adults. Intervals of 10 and 30 min, 1, 2 and 4 hr and 1 day were given between 4 fractions. A 2-day interval was also used for adults. Pupae were not examined in this experiment.

TABLE 3

The effect of varying the interval time on fertility of eggs, larvae and adults

Interval time	Progeny per parent as a percentage of control		
	Eggs (4 × 585 rep)	Larvae (4 × 918 rep)	Adults (4 × 1627 rep)
Continuous treatment	12·9	1·0	1·1
10 min	2·5	2·5	1·1
30 min	17·8	0·0	1·3
1 hr	15·1	32·3	0·8
2 hr	25·0	46·6	0·9
4 hr	12·2	54·8	0·7
1 day	15·1	47·4	0·6
2 days	—	—	4·3

The extent of recovery between treatments is reflected in the increase in survival and fertility with increasing interval. Recovery of somatic cells was rapid and almost complete in 4 hr (Fig. 3). Increasing intervals produced only slight differences in the fertility of eggs, and no recovery of germ cells in irradiated adults (Table 3). Larvae, however, showed a marked increase in fertility with intervals of 1 hr and longer.

The effect of the number of fractions

Changes in susceptibility afforded by varying the number of fractions were tested with adults, using two doses – 6510 and 7500 rep divided into 2, 3, 4 and 5 fractions applied at 24 hr intervals. Survival increased markedly with the number of fractions (Fig. 4); recovery was most marked during the first three intervals (up to about 70 per cent survival), becoming progressively diminished with further subdivisions of dose.

The effect of interval temperature

It is recognised that within certain limits, rate of metabolism increases with temperature. To investigate the effect of temperature on the recovery process, adults were irradiated with 6510 rep by continuous treatment and with

the dose divided into two and four fractions applied at intervals of 24 hr. Irradiations were carried out at 26 °C; interval temperatures were 1°, 10°, 15°, 26° and 30 °C, and all samples were retained at 26 °C after irradiation.

At low temperatures, trends in survival (Fig. 5) were not consistent; with four fractions there was a decrease in survival with increasing temperature from 1° to 15 °C, and a marked increase in survival from 15° to 30 °C. With two fractions, the initial decrease in survival was followed by an increase from 10° to

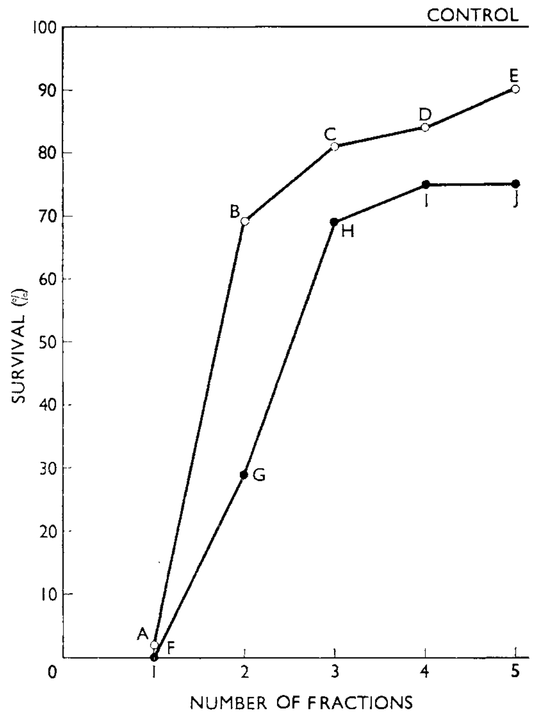


FIG. 4. Survival of adults 60 days after irradiation with fractional doses at 1-day intervals.

○ Total 6510 rep	● Total 7500 rep
A = 1 × 6510 rep	F = 1 × 7500 rep
B = 2 × 3255	G = 2 × 3750
C = 3 × 2170	H = 3 × 2500
D = 4 × 1627	I = 4 × 1875
E = 5 × 1302	J = 5 × 1500

30 °C. There was no significant difference in survival between controls held during the intervals at 1 ° and 30 °C or at 1 ° and 15 °C. As previous experiments had indicated no effect of fractionation on recovery of fertility in irradiated adults, progeny counts were confined only to some of the treatments involving four fractions: with the continuous dose at 26 °C and tempera-

tures between fractionated treatments at 15°, 26° and 30°C the fertility of the irradiated insects (progeny per parent) was 1·1, 0·9, 0·6 and 1·0 per cent of the control, respectively. Accordingly it may be concluded that the interval temperature has no effect on the susceptibility of the gonads.

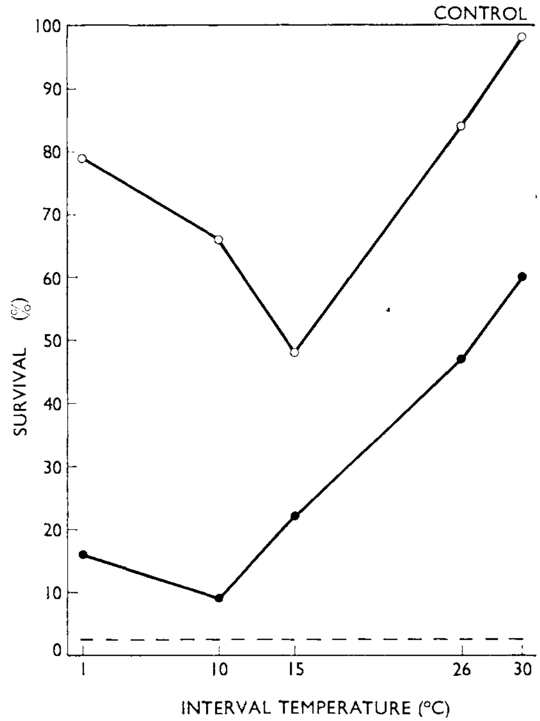


FIG. 5. Survival of adults 60 days after irradiation in relation to the interval temperature in fractionated treatments. The survival level with a continuous dose of 6510 rep is shown by a broken line.

○ – 4 × 1627 rep
● – 2 × 3255 rep

The effect of temperature during irradiation

As the rate of recovery of body cells varies with the interval temperature, irradiation over several hours at a low dose rate and at high and low temperatures should show the presence of recovery during the irradiation period. Adults 28–29 days old were irradiated with 4500 rep at 450 rep/hr. Fifteen samples of 50 insects were treated at both 7° and 29°C. Controls were also retained at 7° and 29°C during the 10-hr irradiation period. Fertility was tested during an oviposition period of 1 month.

Percentage survival, compared with controls, 60 days after irradiation was 102 ± 3 per cent, and 78 ± 3 per cent, at 29° and 7°C, respectively; the dif-

ference is highly significant ($P < 0{\cdot}1$ per cent). Differences in fertility ($2{\cdot}1 \pm 0{\cdot}2$ and $1{\cdot}9 \pm 0{\cdot}1$ progeny per parent) were not significant. There was no significant difference in survival or fertility between untreated controls retained at the two temperatures during irradiation. Thus, recovery of somatic cells also progresses during irradiation at low dose rates, particularly at high temperatures.

The effect of two fractions and increasing intervals

The final experiment was devoted to investigating the amount of recovery in a single interval ranging from 10 min to 10 days. Adults 0–11 days old were treated as follows:

Dose (rep)		Time interval
Fractionated treatments:	2 × 4100 = 8200	4 hr, 1, 1·5, 2, 3, 4, 5, 7, 10 days
	2 × 3750 = 7500	4 hr, 1, 1·5, 2, 3, 4, 5, 7, 10 days
	2 × 3255 = 6510	10, 30 min, 1, 2, 4 hr, 1, 1·5, 2, 3, 4, 5, 7 and 10 days
	2 × 3500 = 7000	1 day
	2 × 3000 = 6000	1 day
	2 × 2500 = 5000	1 day
Continuous treatments:	8200, 7500, 6510, 4100, 3750, 3500, 3255, 3000 and 2500 rep.	

Survival in the first three treatments, 60 days after irradiation, gradually increased with interval time except at 2 days when in all cases it was lower than at 1·5 days (Fig. 6). There was no further increase in survival above the level afforded by one dose. Thus insects which survive the first fractional treatment, and are held for a sufficient interval before the next one, are able to withstand a further dose of the same magnitude with no further mortality attributable to radiation. Complete recovery from a dose of 3255 rep is obtained in about 6 days; larger doses require longer intervals.

Survival after treatment with 2 × 3500, 2 × 3000 and 2 × 2500 rep, and with 1-day intervals, was 49, 74 and 91 per cent, compared with 89, 90 and 97 per cent with single doses of 3500, 3000 and 2500 rep, respectively. Survival after treatment with 2 × 3255 rep with intervals of 10 and 30 min and 1 and 2 hr was 5, 6 9 and 8 per cent, respectively, compared with 0 per cent after a continuous dose of 6510 rep. Thus an interval of only 10 min is sufficient to increase survival above that obtained with continuous treatment.

It can be shown from the data that the observed variations in survival cannot be attributed to changes in overall dose rate; thus with 2 × 3255 rep given to adults with one interval of 4 hr (overall dose rate: 6510 rep in 4 hr 39 min = 1400 rep/hr), 23 per cent survived. On the other hand, 50 per cent were able to survive 4 × 1627 rep with 3 intervals of 1 hr (overall dose rate: 6510 rep in 3 hr 39 min = 1784 rep/hr).

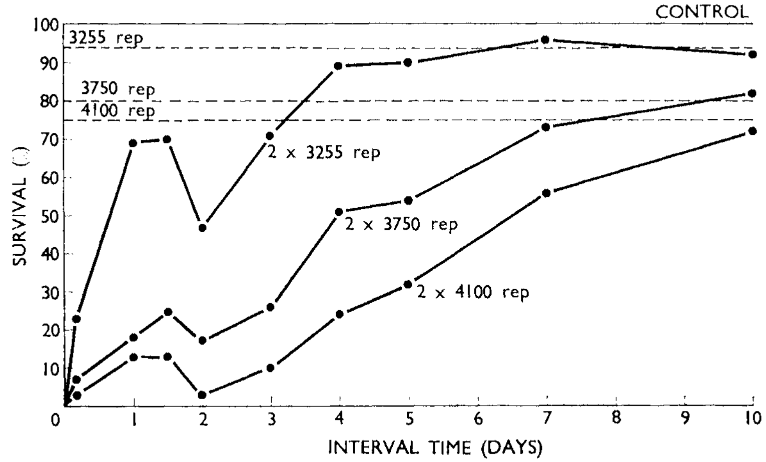


FIG. 6. Survival of adults given two fractional doses with increasing interval time. The survival level for one dose is shown by a broken line.

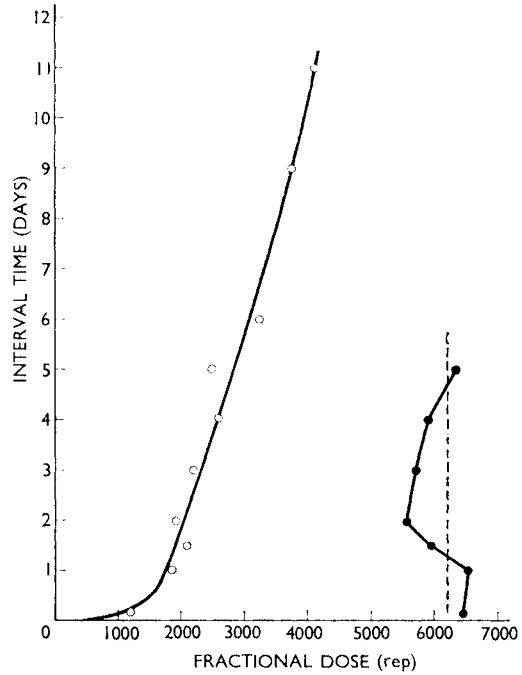


FIG. 7. The relationship between fractional dose and time for maximum possible recovery between two doses. Also the fractional dose plotted against interval time providing the same radiation damage as continuous treatment (i.e. no possible recovery in the interval between two fractional doses).

O – Maximum recovery

● – No recovery

Using the data from this experiment and those previously described, an attempt was made to calculate (Fig. 7) the relationship between dose, which allowed the maximum recovery in adults in the shortest possible time, before the insect could again receive the same treatment and not show an increased mortality. The values for this curve were derived by equation for the intervals of 4 hr to 5 days and by inspection of Fig. 6 for the intervals, 6 to 11 days. Fig. 7 suggests that insects which survive doses of 4000 to 5000 rads would require an interval of almost 2 weeks if they are not to be further reduced in numbers by a second treatment. Additional calculations (Fig. 7) also show the dose from which no recovery is possible. Results suggest that 6200 rads is the upper limit for recovery of somatic cells from radiation damage.

DISCUSSION

Few previous workers have used insects to compare the effects of small repeated doses of radiation with large continuous doses of the same magnitude. The life span of *Tribolium confusum* has been extended with small daily doses of X-rays[6,7] and gamma rays.[8] The dose for sterility in *Trogoderma sternale* has been increased by fractionation[9] and greater emergence obtained from eggs of *Bombyx mori*.[10] Divided doses of X-rays given to *Habrobracon juglandis* provided only temporary sterility.[11]

The experiments reported here have shown differences in the radiation susceptibility of developmental stages of *S. granarius*, when subject to continuous and fractionated treatments, with regard to both survival and fertility. These differences must be attributed to recovery of the somatic and reproductive cells. Recovery may be brought about by repair at the molecular level or replacement at the cellular level. Radiation may damage important macromolecules or disorganise the grouping of molecules in functional units.[12] In the intervals between damage, macromolecules may be repaired and the units reorganised, or new cells may take over the function of damaged cells if cell division is sufficiently rapid. Both repair and replacement would reduce the overall effect of radiation damage and it is probable that both mechanisms are in operation. Repair and replacement probably continue throughout irradiation as well as between treatments. The amount of recovery and level of survival are dependent on the length of the intervals, the fractional dose, the number of fractions and the interval temperature.

Differences between continuous and fractionated doses are apparent with intervals as short as 10 min. Rate of recovery is highest during the first few hours after irradiation and decreases as damage is repaired (Figs. 3 and 6). With low fractional doses (1627 rep in adults) repair is complete in 2 hr; the survival afforded by a 1 or 2-day interval is not much higher than with 4 hr (Fig. 3). With high fractional doses (3255 rep in adults), all damage is too great to be completely repaired within 4 hr and the rate of recovery does not

substantially decrease until 4 days after irradiation (Fig. 6). Increasing the interval between two treatments produces no further recovery when survival has reached the level obtained with one fractional dose. Thus, insects capable of surviving the first fractional dose and given long enough to recover completely would then be able to withstand a further dose of the same magnitude without further mortality attributable to radiation. The three survival curves in Fig. 6 indicate that the higher the dose, the longer the time necessary for recovery; approximately 6 days are needed to recover from 3255 rep, 9 days from 3750 rep and 11 days from 4100 rep. These values are used (with others derived by probit analysis) in Fig. 7 to show that insects capable of surviving a large dose would require extremely long periods so as to survive a second similar dose. The curve would suggest that complete recovery is impossible from a dose greater than 5000 rep, however long the interval between the two treatments. Figure 7 also relates interval time and the calculated fractional dose which provides the same amount of radiation damage as a continuous treatment (i.e. results in zero recovery). It would suggest that two doses of about 6200 rep separated by intervals up to 5 days allow no recovery; survival is equal to that obtained with a continuous dose of 12,400 rep.

An increase in survival is expected with subdivision of treatment into a greater number of smaller fractions, retaining the same interval time, since this reduces the amount of damage to be recovered in each interval. The high rate of recovery exhibited between the first and second fractions is not, however, continued between the second and third, and is still lower between the third and fourth (Fig. 4). These additional treatments may damage newly recovered tissue and impose greater demands on the recovery process which cannot be met.

The present work has also shown that the interval temperature is an important factor influencing recovery. Lamarque,[10] irradiating eggs of *Bombyx mori* with two doses, obtained recovery during an interval at 21 °C; when, however, the eggs were kept "in a refrigerator" the effects of the two fractions were strictly cumulative. Cook,[13] on the other hand, found that eggs of *Ascaris equorum* exhibited greater recovery when retained at 5 °C after irradiation than at 25 °C. The results for *S. granarius* differ from both these observations: survival was greater with fractionated than with continuous treatment at all temperatures, 1°–30°C (Fig. 5); recovery and survival increased with interval temperatures from 15° to 30°C and from 10°C to lower temperatures. Since damage by irradiation is compensated by recovery and the rates of both processes appear to be temperature dependent[14] a balance of the two might be expected to produce differences in survival at different temperatures.

In detailing the results, attention has been drawn to the differences between stages in their capacity to recover from radiation damage; this capacity increases as the insect develops from egg to pupa with a de-

crease after the emergence of the adult. It is plausible to expect the capacity to recover, of insects passing from one developmental stage to the next during the interval between treatments, to be similar to that of stages on either side of the moult. The capacity for recovering fertility also increases with immature development, but is absent in the adult stage. It is of interest to use the data of Table 1 to calculate the dose for 50 per cent reduction in progeny as a percentage of the LD 50/60:

	50 per cent Sterilising dose as a percentage of 50 per cent Lethal dose	
	Continuous treatment	Fractionated treatment
Eggs	92	102
Larvae	78	72
Pupae	31	31
Adults	32	20

Similarity between the scores for continuous and fractionated treatments shows, with the exception of adults, that recovery in the capacity for reproduction occurs to the same extent as recovery in somatic tissues. There was no indication of fertility being regained in later months by adults receiving fractionated doses as found in *Habrobracon juglandis.*[11]

The recovery peak shown by adults at 1·5 days (Fig. 6) is similar to that obtained by other workers treating widely different material. Cell lines of ovarian tissue and female lung tissue of the Chinese hamster, *Cricetulus griseus*, have been irradiated[15] with two doses separated by intervals up to 25 hr; the results were similar to those of the present work except that a peak was obtained with an interval of 2 hr followed by a trough at 4 hr. Pollen grains of *Tradescantia bracteata* treated with two doses separated by intervals of 2–18 hr[16] resulted in fewer chromosome breaks as the interval increased to 4 hr; at 12 hr there was an increase in the number of breaks followed by a decrease again with longer intervals. Similar results are reported by Haque[17] with pollen grains of *Tradescantia paludosa* and by Jain *et al.*[18] with barley root tips. Lane[16] has suggested that the drop in breakage frequency might be attributed to a reduction in sensitivity of the cell to subsequent damage – a radiation-induced protection which disappears with time. With *S. granarius* (Fig. 6) the three peaks certainly cannot be explained by fluctuations in radiosensitivity with age, since the susceptibility of adult weevils remains relatively constant over the first 10 days.[2] Adults receiving four fractional doses did not show this recovery peak with the trough at the 2-day interval, possibly because the fractional doses were low and a much reduced recovery-rate is

observed with an increased number of fractions. There are, however, indications of a recovery peak and trough with eggs and larvae, the trough for these two stages occurring with intervals of 1 hr and 30 min respectively (Fig. 3).

It is pertinent to examine the results of this investigation in relation to radiation disinfestation as an industrial process. Cornwell[2] has derived a dose of 16,500 rep for the commercial treatment of grain, effective against all stages of *S. granarius*. Adults require the highest dose to kill and sterilise with both continuous and fractionated treatments; they are potentially the most dangerous stage in the event of inefficient control. Calculations from regression equations show that less than 1 in 10,000 adults can survive a continuous dose of 16,500 rep. It has also been shown that when a dose is divided into two fractions, with fractional doses of 6200 rep and above, no recovery is apparent with intervals up to 5 days. Moreover, survival is only increased to 1 in 1000 adults when a dose of 16,500 rep is divided into 4 fractions with 1-day intervals at 26 °C. With shorter intervals and lower temperatures, survival is less. Finally, the level of sterility in adults afforded by fractionated treatment is equal to that with continuous doses. It may therefore be concluded that any fractionation likely in practice would not reduce the efficiency of radiation disinfestation of grain. It may in fact be of considerable advantage. If, for instance, irradiation were used to treat grain at intake into a terminal elevator, during operations which normally involve excessively high handling rates, a dose as low as 6000 rep could be given as a preliminary treatment; this would accommodate the high intake and reduce fertility to less than 1 per cent.[2] On the other hand, with low-dose applications to packaged cereal commodities, specifically to prevent the emergence of immature stages, fractionated treatment could seriously jeopardize the efficiency of control.

CONCLUSIONS

1. Survival of all developmental stages of *S. granarius* is significantly increased by dose fractionation, particularly with larvae and pupae, attributable to recovery during the intervals between radiation damage. The amount of recovery and its effect on survival are governed by the number of fractions, the fractional dose, interval time and interval temperature, but not by the overall dose rate. Rate of recovery is greatest in the first few hours after irradiation, and decreases with increasing number of fractions. Intervals of 10 min are sufficient to modify the insects' response. Irradiation is followed by a period of reduced sensitivity to subsequent treatment.
2. Recovery is obtained in the reproductive capacity of irradiated eggs, larvae and pupae, but not adults. Adults subjected to fractionated treatment do not redevelop fertility in later months. Pupae show the highest capacity for recovery of somatic and reproductive cells.

3. Adults require the highest dose to kill and sterilise by continuous and fractionated treatment and are potentially the most dangerous stage in the event of inefficient control. Comparison of the effects on adult survival of continuous and fractionated treatments at 16,500 rep would indicate no loss in the efficacy of radiation disinfestation of grain involving any system of multiple treatment likely to be used in practice. Fractionation could, however, lead to inadequate control of insects in packaged cereal commodities given low doses to prevent adult emergence.

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PAPER V

The Effect of Culture Environment on the Susceptibility of *Sitophilus granarius* (L.) to Gamma Radiation

J. O. BULL AND P. B. CORNWELL

An examination is made of the effects of 8 culture densities and 2 post-irradiation treatments on the susceptibility of adult grain weevils to gamma radiation from ^{60}Co. The lethal effect of gamma radiation is increased by culture densities which cause a substantial rise in metabolic temperature above 26°C during larval development. Densely crowded cultures in which temperatures during growth and maturation of larvae do not rise appreciably above 26°C fail to modify the adult's lethal response. Temperature fluctuations up to 36°C during development do not modify the susceptibility of the adult to radiation sterilisation. Accordingly, the efficacy of 16,000 rads evaluated for the control of grain weevils is unlikely to be reduced by population densities normally encountered in commercial storage.

INTRODUCTION

The need for precise standardisation of technique in the assay of insecticides is now well recognised. Toxicological studies involve two components, the insect and the poison; both must be controlled as far as possible in order to obtain repeatable results.[(1)] Biological variability comprises intrinsic factors, e.g. sex, age and size of the insect, and extrinsic environmental factors, e.g. temperature, humidity, type of food and population density. In both instances a number of factors may interact. For example, population density may have several indirect effects; insects from crowded cultures may be smaller in size, and through continual disturbance of each other, may have a higher metabolic rate *(loc. cit.)*. They may affect the temperature of the culture, or the sex ratio of the populations to be tested. Environmental factors can be further subdivided into those which operate whilst the insect is being reared, and those which operate during and after exposure to the insecticide.

In reporting tests on the susceptibility of insects to ionising radiations little account has been given of the methods of rearing samples for irradiation. With stored products pests it is usual to maintain adults for oviposition on a

standard quantity of food in standard-sized jars for a specified time. This method, used by Cornwell[2,3] when examining the susceptibility of the grain weevil (*Sitophilus granarius* (L.)) to gamma radiation, showed that 16,000 rads produces complete sterility and causes 100 per cent mortality of adults in 2 to 3 weeks. In more recent experiments,[4] populations of weevils were tested for susceptibility when reared in large numbers in paper sacks. Preliminary results indicated that culture environment might have a considerable effect in modifying the susceptibility of the insect to radiation.

The present experiments were designed to evaluate the effects on radiation susceptibility of rearing the grain weevil under a variety of culture conditions. It is difficult in the laboratory to simulate the natural environment of a pest in storage. Nevertheless, it was hoped from these experiments to assess whether a dose evaluated for effective control might be influenced in practice by the degree of infestation of the commodity to be treated. The effects of two experimental environments after irradiation were also examined. Death of the adult and degree of sterilisation were used as criteria of radiation damage.

EXPERIMENTAL

Adult weevils of a laboratory strain were placed to oviposit for 3 weeks on 7 kg of Manitoba wheat in two-ply Kraft paper sacks (17 × 30 in.). Adults of the following generation were used to inoculate eight treatments ranging in density from 1 to 1000 adults per 800 g of wheat (Table 1). They were placed to oviposit for 28 days in jars (5 in. diameter) and paper sacks, in a room at 26 °C and 75 per cent r.h.

In treatments involving fewest adults (4 and 8) equal numbers of males and females were used and the cultures duplicated. All treatments were replicated three times; the first was used to examine changes in temperature, total yield of

TABLE 1

Culture treatments

Treatment	Container	Number of adults	Weight of grain (g)
1		1000	800
2	Jars	250	800
3		100	800
4		10	800
5		1000	800
6	Sacks	1000	8000
7		100	8000
8		10	8000

progeny and their mean weight, the second was sieved periodically to study adult emergence, and the third provided adults for irradiation. These were removed 9 weeks after inoculation and samples of 100 were irradiated over a range of 12 doses (1500–7000 rads) at intervals of 500 rads. Duplicate samples were used for controls. Few adults were obtained from Treatments 4 and 8, and samples were irradiated only at alternate dose levels.

Irradiations were carried out at 26°C in a ^{60}Co source with standard exposure times of 1 and 2 hr for the dose ranges 1500–4000 rads and 4500 to 7000 rads, respectively. During irradiation, samples were contained in small plastic tubes with perforated tops, after which each sample was split into two batches of 50 adults, one incubated on 100 g of wheat in jars (3 in. diameter), the other on 1000 g in jars (5 in. diameter). They were examined for mortality 11, 14, 28, 42 and 56 days later, the surviving insects at 28 days being transferred to fresh grain. The effect of culture environment on the radiation susceptibility of males and females was determined by sexing all insects which died within 42 days of irradiation. Progeny obtained from eggs laid in the 8-week period after irradiation were also recorded.

RESULTS

Culture temperatures induced by insect metabolism

Grain temperatures were measured by a thermocouple placed in the centre of each culture. There were only slight differences between treatments in the first 2 weeks (Fig. 1). During growth and maturation of larvae the temperature of Treatment 1 (the highest concentration in jars) rose to a maximum of 10°C above ambient, and increases in other treatments in jars were roughly proportional to adult density. The temperature rise during larval development in sacks was very much lower. In Treatment 1, the temperature began to fall at about 3½ weeks concurrent with pupation, the fall becoming more marked with the removal of adults at 4 weeks, and continuing throughout the remainder of the experimental period because of the very unfavourable conditions for further insect development. In contrast, the temperatures in Treatments 2, 3 and 6 rose between 7 and 9 weeks concurrent with the development of mature larvae of the following generation. Figure 1 illustrates that temperature fluctuations were not simultaneous in all treatments, indicating a faster rate of development in the dense cultures than in those which were less crowded. In Treatment 1, mould developed on the grain at a very early stage; in Treatments 2 and 3, growth of mould was particularly accelerated during the eighth and ninth weeks, but there was no microfloral activity in Treatment 4 nor in any of the cultures retained in sacks. The mean temperature above ambient for the three periods 0–28, 28–45 and 45–63 days after inoculation of adults is shown in Table 2. These periods correspond

respectively with (1) immature development up to the pupal stage of the first generation, (2) adult emergence and egg-laying, and (3) immature development of the following generation.

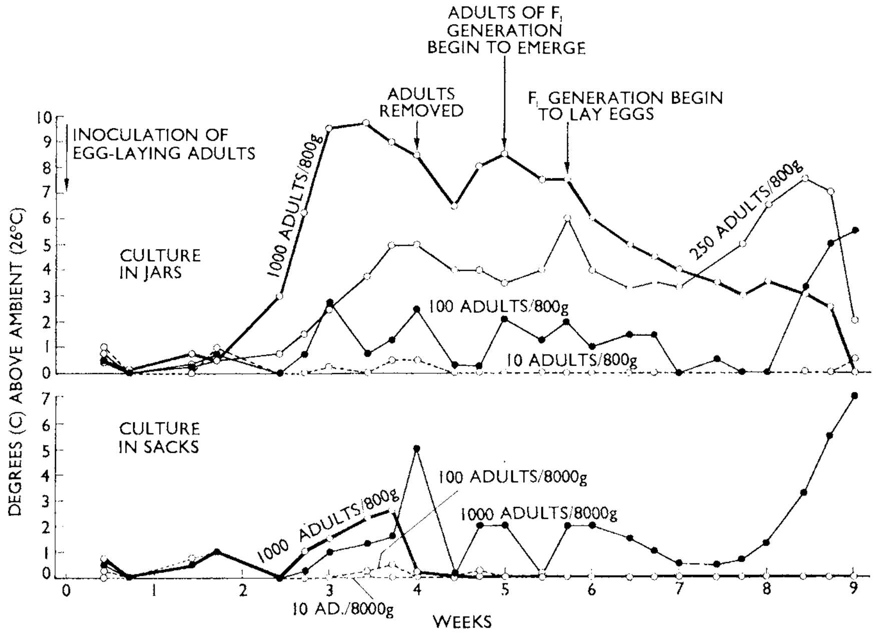


FIG. 1. Changes in temperature of the grain in the eight culture treatments.

Biological effects of the treatment environments

Yield of progeny

The mean number of progeny per parent (Fig. 2a) decreased in both jars and sacks with increase in parent density; it was not possible to test the differences statistically, but it is apparent that the yield in jars was higher in a number of treatments than in sacks at a corresponding density.

Mean weight of progeny

Where possible, 10 samples each of 100 adults were weighed when 0–28 days old. In jars, a maximum weight of adults (2·83–2·84 mg) was obtained in Treatments 2 and 3 (Fig. 2b), and was significantly higher than in Treatments 1 and 4 ($P < 1$ per cent – 0·1 per cent). Heavier adults from Treatments 2 and 3 may have been caused by a higher water-vapour content as reflected by development of mould; the decreased weight of Treatment 1, however, is anomalous and may have been caused by the very unfavourable

conditions for development. The weight of adults obtained from sacks fell with increasing culture density from 2·88 mg in Treatment 8, to 2·64 mg in Treatment 5 ($P < 0·1$ per cent).

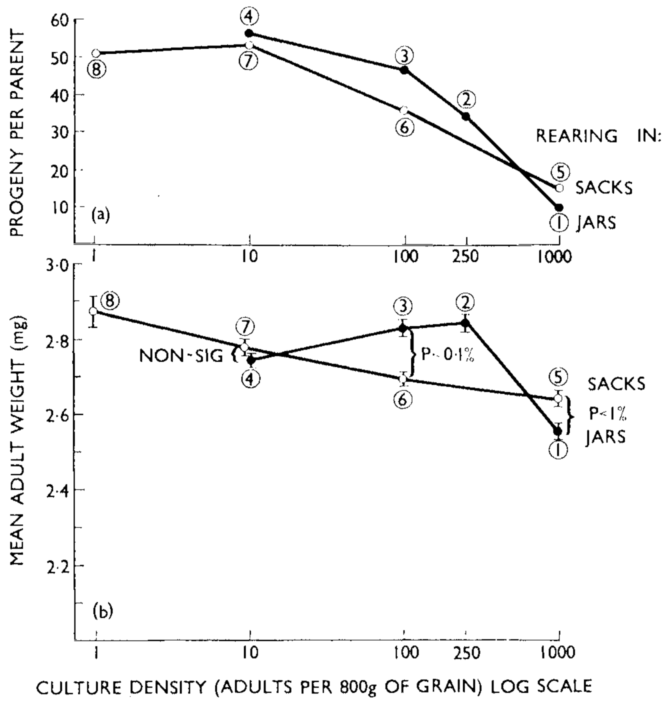


FIG. 2. The effects of culture density (treatments 1–8) on (a) yield of progeny (expressed as progeny/parent) and (b) mean adult weight. Vertical lines denote standard errors.

TABLE 2

Mean temperature (°C) above ambient (26°C)

Treatment	Experimental period (days) 0–28	28–45	45–63
1	4·8	6·9	3·0
2	2·1	4·1	4·8
3	1·0	1·2	2·2
4	0·3	0	0·1
5	0·9	0	0
6	1·2	1·3	2·5
7	0·2	0	0
8	0·1	0	0

Emergence

Adults first appeared in Treatment 1, 32 days after inoculation, and 3 days later, small numbers began to emerge in the other treatments (Fig. 3). The percentage of adults emerging during 3-day periods reached a maximum 9 days after the beginning of emergence in Treatments 1, 2, 5 and 6, and at 18 days in Treatments 3, 4, 7 and 8. The low numbers obtained from Treatment 1 during the latter half of the emergence period, from 7 to 9 weeks, reflect the lack of uninfested grains available for oviposition. At a density of 100 adults/800 g, the faster rate of emergence from Treatment 6 (sacks) compared with Treatment 3 (jars) can be correlated with slightly higher temperatures (Table 2).

The effects of radiation on adult susceptibility

Survival

Probit analyses showed no differences between the LD 50's of adults retained on 100 g and 1000 g of grain at any period from 11 to 56 days after irradiation. The results for the two types of post-irradiation retention were therefore combined and mortality at 42 days was used to compare susceptibility of the eight culture treatments. Rearing at different densities in jars produced differences in LD 50 of up to 2000 rads ($P < 1$ per cent – 0·1 per cent). Rearing in sacks, however, produced no significant change in susceptibility, although the LD 50 again decreased slightly with increasing density (Fig. 4). Estimates of the dose for 99·9 per cent kill were not significantly different, averaging 10,700 rads for all treatments. It must be borne in mind, however, that the variances at this level of lethality are greater than at the LD 50, reducing the possibility of demonstrating differences between treatments.

Susceptibility of the sexes

Before examining the susceptibility of males and females to irradiation, preliminary analyses were carried out to establish whether the culture treatments themselves had produced differences in the sex ratio. Analysis by the chi-squared test (Table 3) of the numbers of males and females in samples of about 400 adults showed that the populations were biased heavily in favour of males ($P < 1$ per cent). Individual tests showed that the numbers of each sex in Treatments 1 and 2 did not deviate significantly from unity, but Treatments 3–8 had a significant preponderance of males ($P < 5$ per cent – 0·1 per cent). A further test for heterogeneity with the exclusion of Treatments 1 and 2 showed that the preponderance of males was common to all Treatments 3–8 ($\chi^2 = 5{\cdot}14$; $P = 50$–30 per cent), the sex ratio, males to females, averaging 1:0·75.

The effect of culture density on the susceptibility of the sexes to irradiation was therefore examined by summing separately the mortalities in Treat-

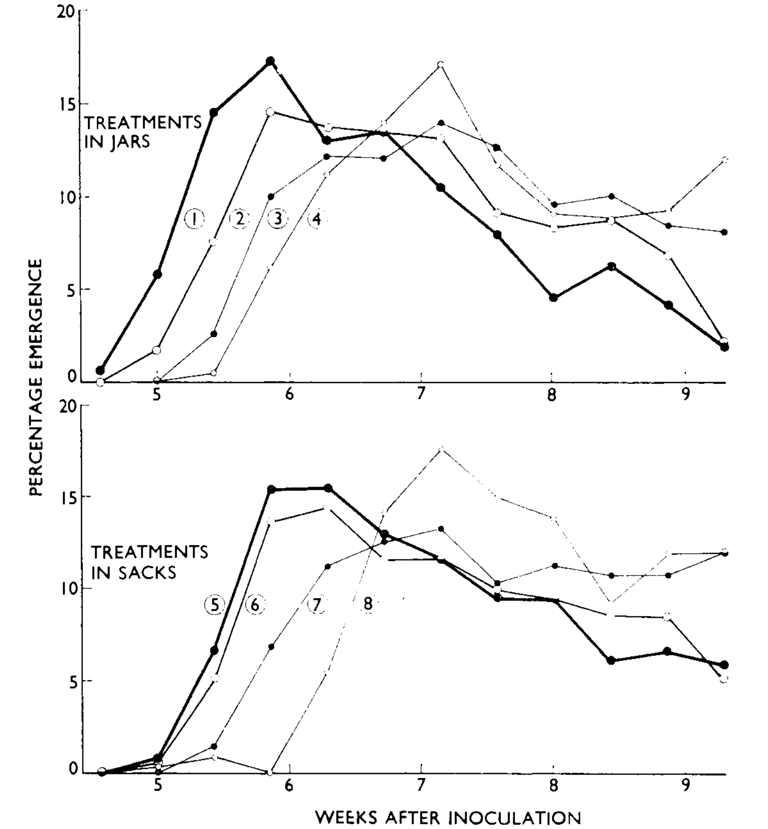


FIG. 3. Percentage of adults emerging in 3-day periods from the eight culture treatments.

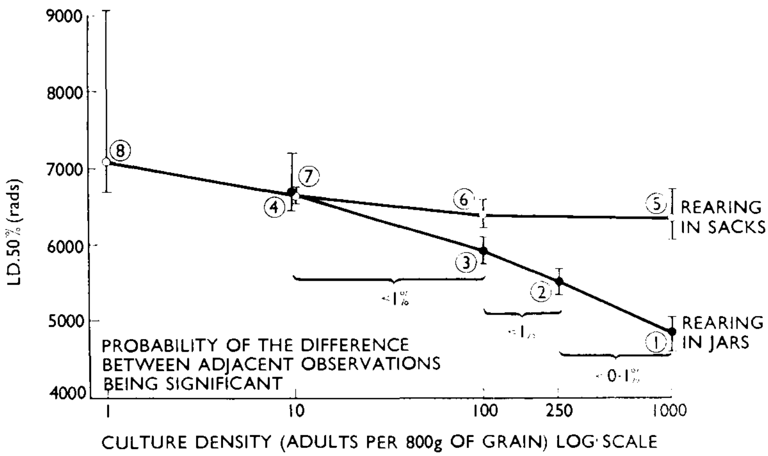


FIG. 4. The effects of culture density (treatments 1–8) on the dose for 50 per cent kill, 42 days after irradiation. Vertical lines denote 95 per cent confidence limits.

ments 1 and 2 and 3–7* for each dose level (Fig. 5), and correcting the observations for differences in population sex ratio and mortality in controls. Because few adults were killed at low doses, the numbers for 1500 and 2000 rads were combined. At doses between 1500 and 4500 rads correction

TABLE 3

Chi-squared analyses on the numbers of male and female adult grain weevils sampled from the eight culture treatments

Test for heterogeneity

Culture treatment	males	females	Contribution to χ^2
1	202	210	6·69
2	257	246	3·72
3	244	184	0·47
4	218	147	2·80
5	230	187	0·01
6	271	226	0·14
7	270	176	4·84
8	271	207	0·34
Total	1963	1583	19·01 $P < 1\%$

Tests for deviation from a sex ratio of unity

Culture treatment	χ^2	Probability
1	0·12	80–70%
2	1·98	20–10%
3	8·14	<1%
4	13·40	<0·1%
5	4·24	<5%
6	3·90	<5%
7	19·40	<0·1%
8	8·30	<1%

for control mortality gave values for survival above 100 per cent. This was confined almost entirely to females. Above 4000 rads, adults of both sexes from Treatments 1 and 2 were more susceptible than from 3, 5, 6 and 7. Analysis of variance of the data for the dose range 5000–7000 rads showed that males of combined Treatments 1 and 2 were significantly more sensitive to radiation (average 18 per cent survival) than females (average 43 per cent survival; $P < 5$ per cent). The apparent difference in susceptibility of

* The incomplete data for Treatments 4 and 8 were omitted.

the sexes in Treatments 3, 5, 6 and 7 (males 61 per cent, females 69 per cent survival) did not, however, approach statistical significance. Clearly, irradiation not only differs in its effect on males and females, but the susceptibility of both sexes is considerably modified by the method of culture (males, $P < 0{\cdot}1$ per cent; females, $P < 5$ per cent).

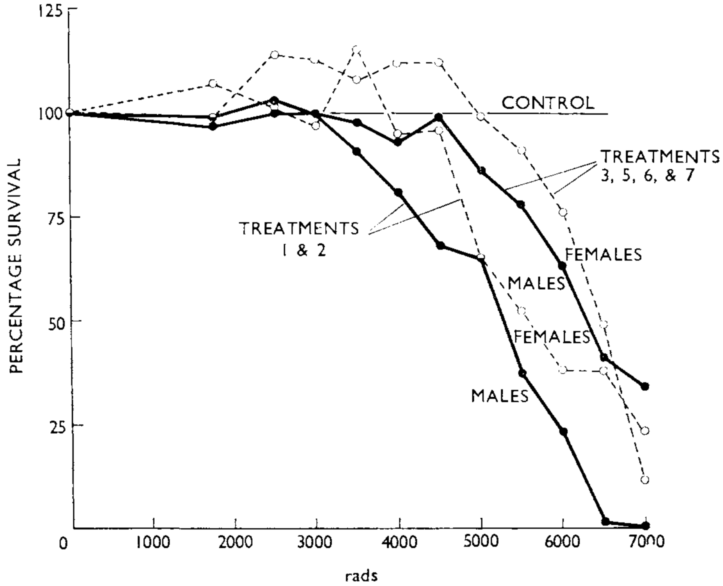


FIG. 5. Effects of radiation and culture treatment on the survival of male and female adult grain weevils. (Data corrected for mortality in controls.)

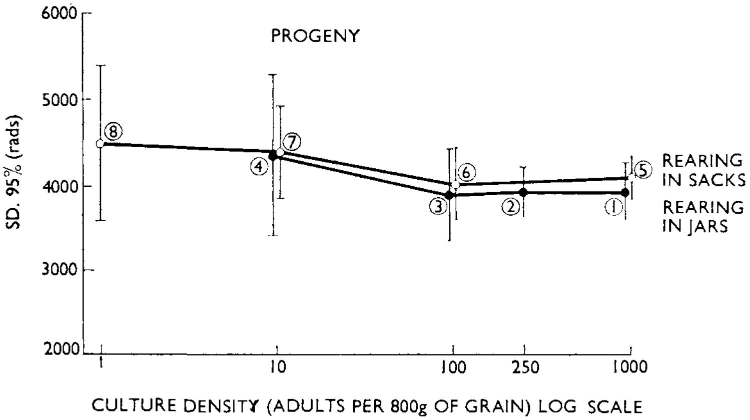


FIG. 6. The effects of culture density (treatments 1–8) on the dose for 95 per cent reduction in progeny. Vertical lines denote 95 per cent confidence limits.

Production of progeny

Retention of adults on two quantities of grain (100 g and 1000 g) after irradiation had little effect on their susceptibility to radiation sterilisation. The results were thus combined and analysed by fitting an exponential function; since culture environment had been shown to modify the susceptibility of adults to the killing effects of radiation, the sensitivity of the insect to sterilisation was examined (1) for progeny and (2) for progeny per surviving parent.

The estimated dose for 95 per cent reduction in progeny (Fig. 6) ranged from 3890 rads (Treatment 3) to 4480 rads (Treatment 8). A similar range was encountered for progeny per parent (4000 rads for Treatment 3 to 4550 rads for Treatment 7), but the differences were not significant for either case. Accordingly it may be concluded that the range of culture densities examined had no modifying effect on the susceptibility of the adult to radiation sterilisation.

DISCUSSION

The effects of culture environment on the susceptibility of insects to chemical insecticides have received little attention. Ishikura and Ozaki[5] report a threefold increase in the susceptibility of the bean weevil, *Callosobruchus chinensis,* to BHC when reared at densities from 50 to 100 adults per 50 g of beans. Sun,[6] however, obtained a greater resistance of *S. granarius* to carbon disulphide with increasing population density. With 50–800 weevils ovipositing on 200 g of wheat for 5 weeks, Parkin (personal communication) found no change in the lethal response of adult *S. granarius* to pyrethrin films. A similar result was obtained with 100 adults ovipositing on 200 g of wheat for 2–17 days. Phillips and Swingle[7] have shown that mortality of mosquito larvae exposed to nicotine and rotenone is significantly increased when the nutrient rearing medium is diluted by half. Commenting on food requirements and insect susceptibility, Potter[8] emphasises that modifications in the response of insects to insecticides, brought about by nutritional inadequacies, are comparable in magnitude to differences in tolerance between resistant and non-resistant strains.

The primary effect of rearing weevils at a high density is to increase culture temperature, particularly of cultures contained in jars. The results of the present study have shown that variations in population density modify the susceptibility of adults to the lethal effect of gamma radiation, but the response to reproductive sterilisation is unchanged. Males are more readily killed by irradiation than females, particularly when reared at high temperatures. Busvine[1] has emphasised that temperature is the primary factor which modifies the susceptibility of insects to chemical insecticides, humidity having little effect.

High temperatures accelerated rate of development. Regression analyses, carried out between LD 50 and the mean temperature above ambient

(Table 2) for three arbitrary periods during incubation, provided good negative correlations ($r = -0{\cdot}94$, $P < 0{\cdot}1$ per cent) for the periods 0–28 and 28 to 45 days (Fig. 7a), but a poor correlation ($r = -0{\cdot}76$, $P < 5$ per cent) for the period 45–63 days. These analyses suggest that the changes in susceptibility may be attributed to the temperature under which the immature stages of the

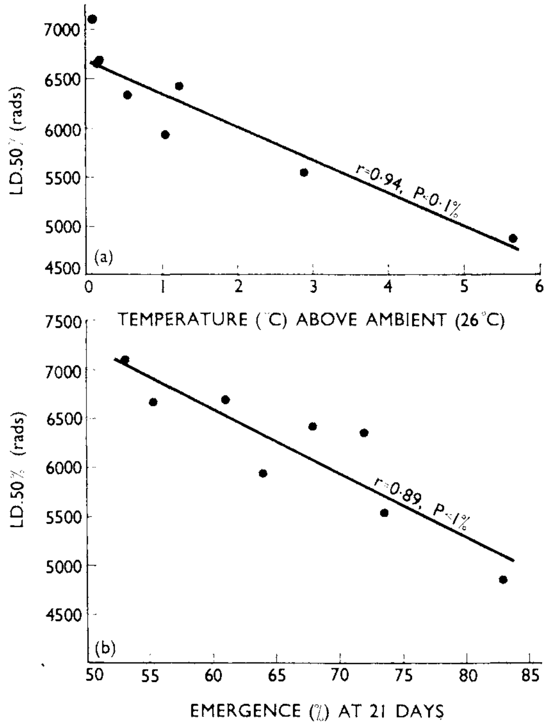


FIG. 7. Relationships between (a) temperature and dose for 50 per cent kill, and (b) rate of emergence and dose for 50 per cent kill.

weevil had developed. The temperatures to which the adults were subjected after emergence, very largely on the surface of the grain, were probably not those measured by the thermocouple in the centre, temperatures around the periphery of the culture being probably much lower. A good negative correlation ($r = -0{\cdot}89$, $P < 1$ per cent) was also obtained between LD 50 and emergence (Fig. 7b). No correlation was obtained between the dose for 50 per cent kill and yield of progeny, or mean weight of adults. Clearly the susceptibility of the adult to irradiation is related indirectly to temperature of rearing, through its effect on the adult body cells.

Pendlebury[9] has already demonstrated that mature adults of *S. granarius*, held before irradiation for 4 weeks at 30 °C, were more readily killed than insects retained at 15 °C. He too found no effect of temperature on production

of progeny, indicating that high temperatures sensitise only the somatic cells and not the reproductive cells. This worker has discussed the possible function of temperature in modifying the lethal response, increased rate of cell division concurrent with high metabolic rate, and an increase in physiological age. The part played by temperature during development of the grain weevil in modifying the susceptibility of the adult cannot be explained without further investigation.

The studies on pyrethrins, communicated by Parkin, are of particular interest since he obtained no modifying effect of population densities similar to those used in the present work: this probably reflects differences in mode of action of the two "insecticides", pyrethrins selective in site of action producing rapid mortality, and irradiation probably causing immediate damage to a variety of tissues, but lethality not becoming manifest until about 2 weeks after treatment.

The higher survival of females at doses below 4500 rads, compared with controls, requires comment; it suggests that female longevity is closely associated with sterility, the production of progeny being reduced to about 5 per cent at this dose. The longevity of female *Ephestia elutella* is extended by 3 or 4 days if unmated, this increase in life-span being attributed to reduced activity and resorption of 40 per cent of their eggs.[10] Increased longevity at substerilising doses has also been observed (unpublished) in a number of species at this laboratory.

Finally, it is pertinent to examine the results of these studies in relation to the sterilising dose of 16,000 rads recommended for commercial disinfestation.[1] The range of doses evaluated for 50 per cent kill is similar to that for 30 wild strains of weevil imported on infested cargoes[2] and the values for 99·9 per cent kill, about 11,000 rads, suggest that the environment of rearing is unlikely to affect the efficacy of radiation in killing weevils at populations even fewer than 10 insects per 8000 g. It can therefore be stated with some confidence that environments found in commercial storage are unlikely to influence the killing effect of 16,000 rads. Moreover, since culture environment did not modify the response to radiation sterilisation, 16,000 rads will be equally effective in controlling infestations of *S. granarius* over the wide range of conditions experienced in commercial practice.

CONCLUSIONS

1. Grain weevils *(S. granarius)* reared at densities ranging from 1 to 1000 adults per 800 g of wheat in jars and paper sacks, increased the temperatures in the centre of the cultures to a maximum during larval development, proportional with population density. Immature development and emergence of adults accelerated with increasing temperature, but the yield of adults and mean weight were depressed at high population densities and could

not be correlated with temperature. A sex ratio of unity was provided by the two treatments which gave rise to the highest temperatures during larval development; the remainder provided a ratio of males : females of 1 : 0·75.

2. The radiation mortality of adults reared in sacks was not modified by population density within the range investigated (1–1000 adults/800 g). The LD 50 for adults reared in jars fell from 6750 to 5000 rads with densities ranging from 10 to 1000/800 g of wheat. Females derived from all treatments were more resistant to killing by irradiation than males. In both densely and sparsely populated cultures, the longevity of females was higher than the controls in the dose range 1500–4500 rads.
3. The susceptibility of adults to radiation sterilisation was unaffected by density of rearing. Susceptibility to killing and sterilisation were unaffected by post-irradiation retention on 100 g or 1000 g of wheat. No loss is envisaged in the efficacy of 16,000 rads for the control of *S. granarius* at densities encountered in commercial storage.

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PAPER VI

Control of Weevil Populations (*Sitophilus granarius* (L.)) with Sterilising and Substerilising Doses of Gamma Radiation

P. B. CORNWELL, J. O. BULL and J. B. PENDLEBURY

The efficacy of 16,000 rads, recommended for industrial applications of gamma radiation for the control of insects in grain, is substantiated by the complete sterilisation and death of 10 million insects of a wild strain of grain weevil under conditions simulating bulk storage.

Substerilising doses of 10,000–14,500 rads suppress weevil populations to a very low level and prevent their increase for 4 to 8 months; this period of "short-term" control is related to the size of the population and oxygen tension at irradiation.

The reproductive potential of fertile and subfertile grain weevils is depressed when added to large irradiated populations; partial protection to the grain against reinfestation is afforded by the insemination of contaminants with sterile sperm, which remains competitive within the female and with fertile sperm subsequently inseminated, for periods greater than 4 months. A reduction in reproductive potential of weevils which may be underdosed at irradiation gives considerable flexibility to the requirement of dose uniformity in plant design for disinfestation of grain.

INTRODUCTION

Using laboratory samples of all developmental stages of an inbred strain of grain weevil (*Sitophilus granarius* (L.)), Cornwell[1] has evaluated 16,000 rads as the minimum dose of gamma radiation for effective sterilisation; it also provides complete kill of all stages, the speed of death of adults depending on temperature.[13] Whilst this dose was found completely sterilising, statistical computations suggest that under the experimental conditions employed, a probability exists, of between 1 per cent and 0·1 per cent, that 16,000 rads could allow a slight measure of fertility. Since a kill by conventional insecticides, at this level of confidence, is acceptable commercially, this dose of radiation has been recommended for future treatment of grain on an industrial scale.

The effective control achieved by 16,000 rads has been further substantiated (Cornwell, *loc. cit.*) with the same strain of weevil, tested in large populations, again under laboratory conditions. Additional support for the use of

this dose commercially has been obtained with the complete sterilisation, in culture, of 30 wild strains of weevil of which more than half were imported on infested cargoes;[2] it is also effective in the control of wild strains of *Sitophilus* and *Tribolium* species naturally infesting cereals in Australia.[3] The question remains, however, as to the effectiveness of this dose against wild strains of weevil, (1) when applied on industrial plant with some measure of accidental or deliberate underdosing, (2) when the grain and insects are retained after irradiation in bulk storage, and (3) when applied to the enormous populations which may develop in commercial storage.

A dose of radiation, which inhibits insect reproduction and produces ultimate death, allows the storage of grain free from infestation for an indefinite period. In commercial practice, however, retention of grain in storage or during shipment may vary from a few weeks to many months. It may be sufficiently short to allow the use of substerilising doses of radiation to inhibit insect multiplication for the required period. That levels of radiation much lower than the recommended dose provide a substantial reduction in fertility is already well known. As the cost of radiation treatment increases with dose, a substerilising dose might prove more attractive economically if milling and consumption of the product follow within a relatively short time after irradiation.

Protection against re-infestation is a prerequisite of storage, if grain is to be maintained in sound condition for any period. With the continued survival of adult weevils for a short time after irradiation, live sterile males may afford some protection to the commodity by the insemination of female contaminants with non-viable sperm. The effectiveness of sterilised males in reducing and ultimately eliminating field populations of screw-worm is already proven.[4,5] The extent to which sterilised males might protect stored products commodities against reinfestation has so far not been examined.

The experiments described here were carried out:

(1) To investigate the effect of substerilising doses on the reproductive potential of different-sized populations of adult grain weevils,

(2) To examine to what extent sterilised populations depress the reproductive potential of smaller numbers of fertile and subfertile weevils, and

(3) To determine the efficacy of 16,000 rads against a wild strain of *Sitophilus granarius* in massive numbers and in all developmental stages under conditions simulating commercial bulk storage.

THE EFFECT OF SUBSTERILISING DOSES ON REPRODUCTIVE POTENTIAL

Three factors are largely responsible for the rate of increase of weevil populations treated with substerilising doses of radiation:

(1) The effects of the environment during irradiation on the lethal and sterilising response.

(2) Survival of sterile and substerile adults after irradiation, frequency of mating and availability of viable gametes.
(3) Favourability of the environment for development.

Among environmental factors at irradiation which modify biological susceptibility, oxygen tension is recognised to be of considerable importance; dominant lethal damage to irradiated immature germ cells of *Drosophila virilis* is enhanced by small amount of oxygen;[6,7] survival of grain weevils irradiated in an oxygen-free medium is three times greater than in air.[8] In grain, the balance between oxygen and carbon dioxide will be influenced by respiration of the seed and by the numbers of insects present. It will be modified by the amount of ventilation in storage and by aeration during irradiation handling. Continued survival of sterile and substerile adults after irradiation will provide a source of non-viable sperm and a carrier for recessive lethal mutations, deleterious to the production of progeny by subsequent generations.[9,10]

Two types of experiment were therefore carried out using substerilising doses. The first with populations of adult weevils, irradiated under conditions of oxygen depletion far more acute than are likely to be met in grain storage practice, to increase the insects' resistance to killing and sterilisation to a maximum. The second, under atmospheric conditions at irradiation. Both experiments were designed to evaluate the rate of increase of different-sized populations using a laboratory strain.

Irradiation under reduced oxygen

In the first experiment, four populations of adult grain weevils, 1000, 10,000, 100,000 and one million, were reared on Manitoba wheat in bins and irradiated at 14,500 rads $\pm$ 2 per cent at a dose rate of 28,400 rads/hr. During irradiation, the adults in batches of 7000–8000 were contained in polythene tubes (24 $\times \frac{1}{2}$ in. diameter) covered by muslin at one end and stoppered at the other. When removed after irradiation, the adults were immobile and suffering from temporary asphyxiation.* Each population was then contained on 4 cwt of Manitoba wheat in a closed tank ventilated with air conditioned to 25 °C and 70 per cent r.h. Temperatures recorded with thermocouples a few inches below the surface of the grain rose sharply in the tank containing one million adults to a maximum of 37 °C, 3 days after irradiation, approaching the limit for lethality† and inhibition of oviposition.[12] From

* Mathlein[11] has shown that adult grain weevils can be immersed under water for 2 weeks without loss of survival.

† Tests to determine the effect of tank environment on mortality showed that with unirradiated populations ranging from 100 to 1·8 million adults, mortality 3 weeks after inoculation ranged from 15–30 per cent in populations of 100–100,000 adults, reaching 50 per cent and 90 per cent respectively in populations of 400,000 and 1,800,000; most deaths occurred in the first week after inoculation. With 3 populations, of 7500, 75,000 and 750,000 adults, treated with 10,000 rads under atmospheric conditions, 30 per cent, 50 per

2 weeks after irradiation the mean temperatures ranged from 25 °C for the lowest population to 28 °C for the highest.

Some live insects could be seen in all populations 5 weeks after irradiation, but at 3 months, survivors could be found only in populations of 10,000 weevils and above. At 4 months, samples of grain constituting about 3 per cent of the bulk indicated 0, 200, 14,000 and 14,500 survivors in populations of

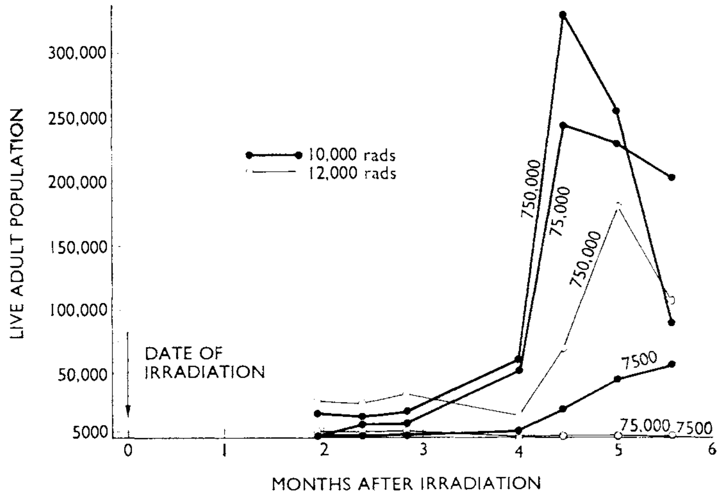


FIG. 1. The effect of substerilising doses (10,000 and 12,000 rads) on different sized populations of adult grain weevils (7500, 75,000 and 750,000) with time, after irradiation under reduced oxygen.

increasing size. At 5 months, evidence of fertility in the tank originally containing 100,000 adults was obtained from incubated samples. Proof of fertility in all populations was not obtained until 8 months after irradiation. It was clear from this experiment that 14,500 rads, although substerilising, is an acceptable dose for controlling weevil populations for 5 to 8 months.

In a subsequent experiment, three populations of adult weevils, 7500, 75,000 and 750,000, were each treated at lower doses, 10,000 and 12,000 rads ± 2 per cent. Irradiations were again carried out under depleted oxygen. Temperatures in the tanks rose above 30 °C during the 2 weeks following irradiation and thereafter ranged from 26 °C to 28 °C in the lowest and highest populations, respectively. Fertility was assessed at intervals over a period of 6 months by inserting 8 metal cylinders into the tanks, removing cores of

cent and 75 per cent mortality was obtained with increasing population 2 weeks after irradiation— not markedly different from the results obtained at 3 weeks with fertile insects— and the corresponding values at 3 weeks were 98·9 per cent, 99 per cent and 98·6 per cent. Rate of mortality thus increased with the size of substerile population, possibly reflecting differences in post-irradiation temperature,[13] but mortality at 3 weeks was unaffected by population size.

grain and counting the numbers of live adults. At the final sampling, all live adults were sieved out and a statistical relationship obtained between sample size and the total adult population. This relationship (log population = 1·116, log sample −0·17) showed a loss in sampling efficiency with increase in population:

Adult population	Efficiency of sampling estimate (%)
100	87·0
1000	69·0
10,000	55·0
100,000	45·0
300,000	36·0

The estimates of the tank populations were then corrected for sampling efficiency.

In all treatments (Fig. 1) the numbers of live adults remained stable for 3 months after irradiation. At 4 months, reproduction was apparent in the two highest populations treated with the lower dose (10,000 rads). At 4½ months, the numbers of live adults began to increase in the lowest population treated with 10,000 rads, and in the largest population treated at 12,000 rads. At 5 months, conditions in some tanks were becoming unfavourable, with the result that the numbers of live adults fell markedly. That reproduction was continuing, but adults were dying soon after emergence, was confirmed by the enormous numbers of dead adults taken in samples. There was no evidence of any increase in the 2 lowest populations treated at 12,000 rads even at 6 months. Clearly, 10,000 and 12,000 rads are adequate for maintaining insect populations at a low level in storage for 4 months; effective control for at least 6 months may be provided by 12,000 rads if the initial population is low.

Irradiation under atmospheric conditions

In a comparable experiment to examine changes in population after irradiation in air, six populations of weevils similar in size to those used in the previous study (7500, 75,000 and 750,000) were irradiated when 3 weeks old, again at 10,000 and 12,000 rads, at a dose rate of 26,000 rads/hr; they were treated in the absence of grain, in large paper sacks, which allowed adequate aeration during irradiation. The adults were again contained in ventilated tanks each holding 4 cwt of Manitoba wheat. Additional adults in 30 batches of 25,000 were also contained on 7 kg of Manitoba wheat in paper sacks to provide an estimate of mortality and fertility; at both dose levels 0·3 per cent survived 3 weeks after irradiation, and at 6 weeks all were dead. Progeny

resulting from eggs laid in the first 3 weeks averaged 117 ± 8 and 13 ± 0·6 per sack at 10,000 and 12,000 rads, respectively. In the second 3 weeks, no progeny were produced. In a control population of 7500 adults, 89 per cent survived 3 weeks and 83 per cent survived 6 weeks; about 70,000 progeny were produced in each period of 3 weeks.

In the tanks, maximum temperatures in the grain supporting the highest populations rose to 40 °C, 5 days after inoculation, and to about 30 °C for the intermediate sized populations; the temperature was unchanged in the tanks containing the 7500 adults. Following these initial high temperatures,

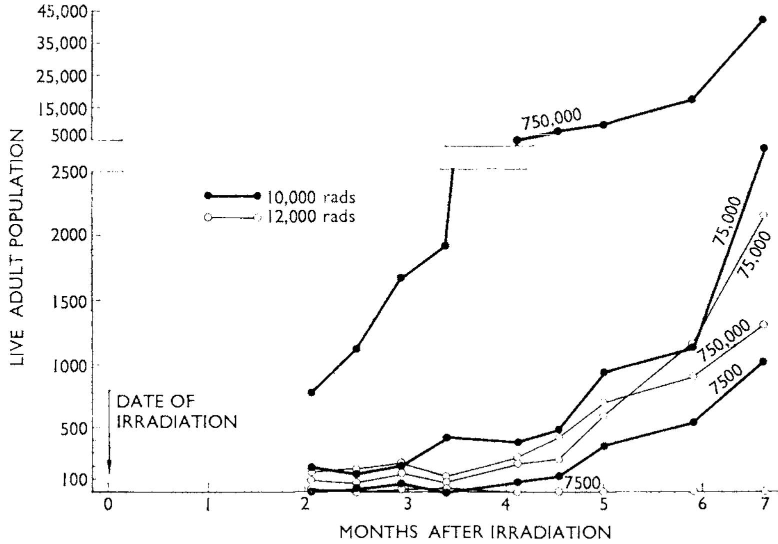


FIG. 2. The effect of substerilising doses (10,000 and 12,000 rads) on different sized populations of adult grain weevils (7500, 75,000 and 750,000) with time, after irradiation under atmospheric conditions.

the thermocouple readings over the remainder of the experiment averaged 25 ° and 24 °C, in the highest and lowest populations respectively. Populations in the tanks (Fig. 2) were estimated over a period of 7 months.* In the tank inoculated with 750,000 adults treated with 10,000 rads, the numbers increased from 700 adults at 2 months to 41,000 at 7 months. In the remaining tanks, less than 200 adults were recorded at 2 months and there was no marked increase in population until about 4 months. No live adults were

* In this experiment, samples from the tanks constituting 5 per cent of the bulk were taken with 20 rectangular cores which allowed a proportion of grain to be taken at the sides where adults tended to congregate owing to metabolic heat of the inoculated population and larvae developing in the grain. The greater number of smaller samples also provided a more reliable measure of population.

found in the tank inoculated with the lowest population treated at 12,000 rads even at 7 months. In this experiment, populations did not reach a level which resulted in unfavourable conditions for further development (cf. Fig. 1).

It is now pertinent to compare the results of the two experiments in which the same sized populations were irradiated under reduced oxygen and atmospheric conditions (Table 1). For the period from 2 to 4 months after

TABLE 1

Average progeny per parent (× 10,000) for the period 2–4 months after irradiation of 3 populations of adult grain weevils treated at 10,000 and 12,000 rads under anoxic and atmospheric conditions

Population	Irradiated under:					
	Reduced oxygen			Atmospheric		
	rads		Means	rads		Means
	10,000	12,000		10,000	12,000	
7500	4026	417	2222	51	6	28
75,000	2657	777	1717	31	18	24
750,000	394	366	380	28	3	15
Means	2359	520		37	9	

irradiation, the average number of progeny per inoculated adult (multiplied by 10,000 to facilitate comparison) shows that treatment under anoxia increased resistance to sterilisation by a factor of about 60. Raising the dose to 12,000 rads, irrespective of the oxygen tension, reduced the number of progeny to a quarter of that obtained at 10,000 rads. Table 1 also shows that with both types of irradiation, the fertility of 750,000 insects was markedly lower than that of the smaller populations – very probably the effect of overcrowding and temperature upon oviposition.

Figures 1 and 2 give the impression that rate of increase is more rapid with treatment of large populations than small ones. Examination of Fig. 2, however, shows that at about 7 months, 7500 adults irradiated at 10,000 rads produced about 1000 insects. With ten times as many adults (75,000) the population at 7 months was only 2800 insects and with 100 times as many (750,000) the population was 41,000 insects. Thus with irradiated populations of the ratio 1:10:100, insects of subsequent generations numbered 1:3:41. The unexpectedly low numbers produced by large populations may be attributed to limitations imposed by the experimental environment. With unlimited amounts of grain the populations would probably have increased proportionately with the numbers irradiated.

PROTECTION BY STERILISED INSECTS AGAINST REINFESTATION

The effectiveness of sterile adults in protecting grain after irradiation from reinfestation by the same species was investigated by adding fertile and subfertile adults in known ratios to large populations treated with 16,000 rads. The effect of sterile sperm on the reproductive potential of the contaminants was assessed by the numbers of progeny. Two experiments were carried out, the first with irradiation under reduced oxygen, shown previously to increase the resistance of weevils to radiation damage, and the second under atmospheric conditions.

Irradiation under reduced oxygen

Large populations of grain weevils of the laboratory strain, reared on Manitoba wheat in cardboard trays, were irradiated, some at 6000 rads, some at 16,000 rads and the remainder retained as controls. Doses were delivered with an accuracy of ± 2 per cent at 13,000 rads/hr. Thirteen treatments were set up (Table 2), comprising "standard" treatments (1–7), in which unirradiated adults and those treated at the two dose levels were maintained independently, and "combined" treatments (8–13), in which unirradiated adults and those treated at 6000 rads were added in three proportions

TABLE 2

Experimental treatments

Treatment		No. Adults	Dose (rads)
Standard	1	20	0 (fertile)
	2	200	
	3	2000	
	4	20	6000 (subfertile)
	5	200	
	6	2000	
	7	10,000	16,000 ("sterile")
Combined (with 10,000 adults given 16,000 rads)	8	20	0 (fertile) with "sterile"
	9	200	
	10	2000	
	11	20	6000 (subfertile) with "sterile"
	12	200	
	13	2000	

as contaminants to 10,000 adults treated at 16,000 rads. Four batches were set up of each treatment on 7 kg of grain in paper sacks at 26 °C and 76 per cent r.h. Two and 6 weeks after irradiation, the surviving adults were transferred to fresh grain and discarded finally at 10 weeks. Fertility was measured by the mean number of progeny per parent for each batch of insects and for each oviposition period.

Survival

In the earlier experiments described, rate of mortality was influenced by size of population; survival in the various treatments of the present experiment were therefore also examined.

In the standard treatments (Table 3) 90 per cent of fertile insects survived 2 weeks after irradiation and 60 per cent survived 10 weeks; these values correspond with 70 per cent and 45 per cent survival of subfertile insects. Differences between concentrations were not significant. About 26 per cent of "sterile" insects survived 2 weeks after irradiation, falling to 3 per cent at 10 weeks.

TABLE 3

Mean survival of adults in the standard treatments (1–7)

Treatment no.	Dose	Concentration	Survival (%) at		
			2 weeks	6 weeks	10 weeks
1	Fertile (unirradiated)	20	81	74	60
2		200	83	78	57
3		2000	98	66	61
4	Subfertile (6,000 rads)	20	60	50	38
5		200	74	66	51
6		2000	84	63	44
7	"sterile" (16,000 rads)	10,000	26	5	3

The observed survival rates in the combined treatments (Table 4) were compared with expected values calculated as follows: 2 weeks after irradiation the expected value for combined treatment 10 was 26 per cent of 10,000 + 98 per cent of 2000 = 2600 + 1960 = 4560 adults = 38 per cent of 12,000 adults. Analysis of variance of the data transformed to angles, showed that at the end of the first oviposition period (2 weeks) the observed survival rates in the combined treatments were slightly (5 per cent), but significantly, higher than the calculated values ($P < 0{\cdot}1$ per cent), caused probably by errors in separating live adults from the large numbers of dead in Treatment 7. Differences between observed and calculated values for survival, 6

and 10 weeks after irradiation, were, however, not significant. Clearly, survival of the contaminants was not reduced by their inclusion within large populations of sterile insects.

Fertility

Treatment with 16,000 rads was incompletely sterilising. Thus, to obtain a value for the mean number of progeny per parent in the combined treatments, it was necessary to derive an estimate of fertility of the contaminants, independent of the low residual fertility of the "sterile" insects. In the first

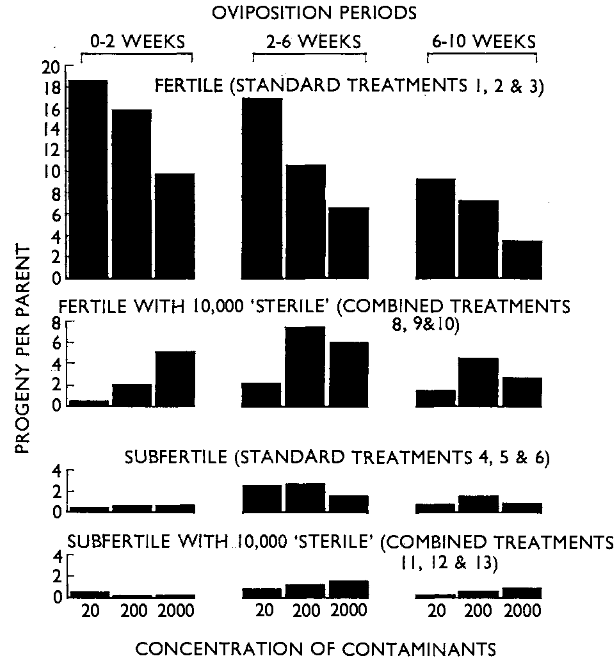


FIG. 3. Mean weekly production of progeny per live parent in the Standard and Combined Treatments. Irradiation under reduced oxygen. Least difference between means for significance at 1 per cent probability = $\pm 3{\cdot}7$ for treatments 1–3 and 8–10, and $\pm 0{\cdot}9$ for treatments 4–6 and 11–13.

oviposition period, 0–2 weeks, the total progeny (Table 5) produced in the four batches of Treatment 8, for example, was 733 compared with 673 by "sterile" insects alone (Treatment 7). The contribution to progeny by contaminants in Treatment 8 was therefore 8·2 per cent. The number of progeny in each of the four batches of Treatment 8 was then corrected to this proportion. These values were then divided by the average number of surviving parents in the appropriate standard treatment – in this instance, Treat-

TABLE 4

Observed (mean) and expected survival of adults in the combined treatments (8–13)*

Treatment no.	Contaminants: Type	Contaminants: Concentration	Survival (%) at 2 weeks: observed	2 weeks: expected	6 weeks: observed	6 weeks: expected	10 weeks: observed	10 weeks: expected
8	Fertile (unirradiated)	20	32	26	10	5	8	3
9		200	32	27	9	7	6	4
10		2000	42	38	15	15	11	13
11	Subfertile (6000 rads)	20	34	26	6	5	4	3
12		200	28	27	4	6	2	4
13		2000	42	35	13	14	11	10
Means:			35·0	29·7	9·3	8·7	6·8	6·2
Probability:			<0·1%		non-sig.		non-sig.	

* Expected survival rates derived from data obtained in the Standard Treatments (1–7).

TABLE 5

Total progeny in three oviposition periods (of 2, 4 and 4 weeks) in the Standard and Combined Treatments. Irradiation under reduced oxygen

Treatment no.		Concentration	Oviposition period (weeks) 0–2	2–6	6–10
1	Fertile standard	20	2658	4216	2059
2		200	22,863	27,072	15,475
3		2000	164,486	188,429	81,269
8	Fertile combined with 10,000 "Sterile"	20	733	548	381
9		200	3712	19,133	9680
10		2000	85,063	172,285	63,225
4	Subfertile standard	20	65	486	109
5		200	1019	6066	2755
6		2000	10,118	40,041	16,376
11	Subfertile combined with 10,000 "Sterile"	20	749	151	100
12		200	851	2737	1224
13		2000	4223	41,975	17,429
7	"Sterile" standard	10,000	673	20	67

ment 1, thus giving the mean progeny per parent (Fig. 3).* They were then examined by analysis of variance, using the variation between batches to test the significance of differences between concentrations, and between the Standard and Combined Treatments.

In the Standard Treatments 1, 2 and 3 (fertile adults alone), the mean number of progeny in each oviposition period was depressed as the concentration of parents increased (Fig. 3). In Standard Treatments 4, 5 and 6 (6000 rads; subfertile adults alone), production of progeny was severely reduced, density of parents having little effect. In the Combined Treatments 8, 9 and 10, progeny production by fertile adults was severely reduced when contained with "sterile" populations, most marked in the first oviposition period when a large proportion of the 10,000 "sterile" insects were alive, and particularly where the contaminants were very much outnumbered (10,000:20, cf. 10,000:2000). In the second and third periods, when the numbers of surviving "sterile" insects had dropped to about 500 and 300 respectively (Table 3), fertility of the 20 and 200 contaminants was still much reduced ($P < 1$ per cent and 5 per cent, respectively); with 2000 contaminants, which themselves outnumbered the surviving "sterile" insects, there was no reduction in fertility compared with the Standard Treatment. The increased reproductive capacity in the second and third periods, compared with the first, may be attributed both to a reduction in the availability of sterile sperm and to some relief from the disturbing effect of sterile insects upon oviposition. Finally, in Combined Treatments 11, 12 and 13, fertility of the subfertile contaminants was only in certain instances reduced by combination with large populations of "sterile" insects; there was no significant effect in the first oviposition period, but fertility of the 20 and 200 contaminants was further reduced to about a third and a half in the second and third ($P < 1$ per cent and < 5 per cent, respectively). Fertility of the 2000 contaminants was unaffected – comparable with the result obtained with fertile contaminants.

A result of particular interest from this experiment is that ratios of 500, 50 and 5:1 do not result in comparable reductions in fertility, explained partly by matings having taken place within the fertile or subfertile populations before the combined treatments were set up. With death of the "sterile" insects, 3 weeks after irradiation, physical disturbance to oviposition and the availability of sterile sperm become much reduced; no reduction in fertility of the 2000 contaminants from the second to tenth week of the experiment, indicates that sterile sperm inseminated in the first oviposition period was having no subsequent effect. Loss of fertility resulting from inseminated sterile sperm in the lower numbers of contaminants, however, (20 and 200) was still manifest 6–10 weeks after irradiation. During this period the contaminants were still outnumbered by live "sterile" insects.

* The results obtained by this calculation are likely to be slightly in error for the first oviposition period since it has been shown that survival rates in the combined treatments were somewhat above those anticipated.

Irradiation under atmospheric conditions

A similar experiment was carried out with irradiation under atmospheric conditions, in which four ratios of sterile insects to fertile and subfertile contaminants were examined (Table 6), made up of different numbers of adults to demonstrate possible disturbance by sterile insects upon oviposition. Equal numbers of males and females were used to make up the populations of 20 or less. Weevils were irradiated at 13,400 rads/hr and retained on 7 kg of wheat, again in paper sacks. Survivors were transferred to fresh grain at 3-weekly intervals for 18 weeks. Progeny were recorded from each oviposition period.

TABLE 6

Numbers of adults in experimental treatments

Treatments	Dose (rads)		
	16,000 (Sterile)	6,000 (Subfertile)	0 (Fertile)
Standard	10,000	12,000	12,000
	1000	10,000	10,000
	100	2000	2000
	10	1200	1200
		1000	1000
		200	200
		120	120
		100	100
		20	20
		12	12
		2	2
Combined	Ratio	Sterile + Subfertile	Sterile + Fertile
	10 : 2	10,000 + 2000	10,000 + 2000
		1000 + 200	1000 + 200
		100 + 20	100 + 20
		10 + 2	10 + 2
	100 : 2	10,000 + 200	10,000 + 200
		1000 + 20	1000 + 20
		100 + 2	100 + 2
	1000 : 2	10,000 + 20	10,000 + 20
		1000 + 2	1000 + 2
	10,000 : 2	10,000 + 2	10,000 + 2

TABLE 7

Mean progeny per live parent in the standard and combined treatments. Also total progeny over the period of the experiment

Combined treatments

Ratio	Dose	Population size	Oviposition period (days) 0–21	21–42	42–63	63–84	84–105	105–126	Total progeny 0–126
10,000 : 2	16,000 + 6000	10,000 + 2	0	—	—	—	—	—	0
	16,000 + 0	10,000 + 2	0	0	—	—	—	—	0
1000 : 2	16,000 + 6000	10,000 + 20	0	0	0	0	0	0	0
		1000 + 2	0	—	—	—	—	—	0
	16,000 + 0	10,000 + 20	5·8	17·7	13·0	4·5	1·4	0	709
		1000 + 2	3·5	41·0	28·0	0	0	0	145
100 : 2	16,000 + 6000	10,000 + 200	0·06	0	0	0	0	0	7
		1000 + 20	0	0	0	0	0	0	0
		100 + 2	0	—	—	—	—	—	0
	16,000 + 0	10,000 + 200	15·7	29·4	9·1	4·8	1·3	0	9300
		1000 + 20	11·8	14·1	8·6	1·08	0	0	620
		100 + 2	2·5	4·5	0	0	0	—	14
10 : 2	16,000 + 6000	10,000 + 2000	0·08	0·01	0·01	0	0	0	99
		1000 + 200	0·09	0	0·06	0	0	0	11
		100 + 20	0·09	0	0	—	—	—	1
		10 + 2	0	0	0	0	0	0	0
	16,000 + 0	10,000 + 2000	23·7	22·3	15·8	12·4	3·0	0·35	108,000
		1000 + 200	23·3	25·7	18·2	6·7	2·4	0·37	12,700
		100 + 20	26·4	32·4	22·6	3·5	1·1	0	1500
		10 + 2	33·5	29·0	30·0	42·0	45·0	19·0	400

Standard treatments									
16,000	10,000	0	—	—	—	—	—		0
	1000	0	—	—	—	—	—		0
	100	0	—	—	—	—	—		0
	10	0	—	—	—	—	—		0
6000	12,000	0·40	0·002	0·04	0·003	0	0		2750
	10,000	0·38	0·005	0·03	0·004	0	0		2210
	2000	0·30	0	0	0	0	0		1330
	1200	0·27	0	0·02	0	0	0		190
	1000	0·35	0	0·03	0·01	0	0		200
	200	0·27	0	0	0	0	0		30
	120	0·24	0	0	0	0	0		15
	100	0·14	0	0	0	0	0		8
	20	0	0	0	0	0	0		0
	12	0	0	0	0	0	0		0
	2	0	—	—	—	—	—		0
0	12,000	10·7	9·7	6·3	4·3	1·6	0·32		317,000
	10,000	11·8	10·7	8·0	7·9	5·1	1·5		347,000
	2000	29·0	20·5	14·6	7·7	2·8	0·97		129,000
	1200	40·0	27·9	24·4	15·1	10·5	4·1		111,000
	1000	38·8	33·8	24·5	16·3	13·4	6·2		105,000
	200	34·7	19·9	14·5	7·1	3·7	0·49		20,000
	120	49·6	38·9	28·5	15·8	11·2	4·3		14,000
	100	45·4	30·5	24·4	13·9	6·7	1·4		9700
	20	57·4	42·9	32·4	21·6	11·3	2·9		2700
	12	56·9	40·4	34·0	20·4	4·7	1·0		1600
	2	60·5	47·5	50·5	36·7	1·0	0		370

Dash signifies total death of parents

Survival

All adults irradiated at 16,000 rads were sterile and died within 3 weeks of irradiation (Table 7). Survivors in the combined treatments were therefore entirely the fertile and subfertile contaminants. Analysis of the data, with percent-

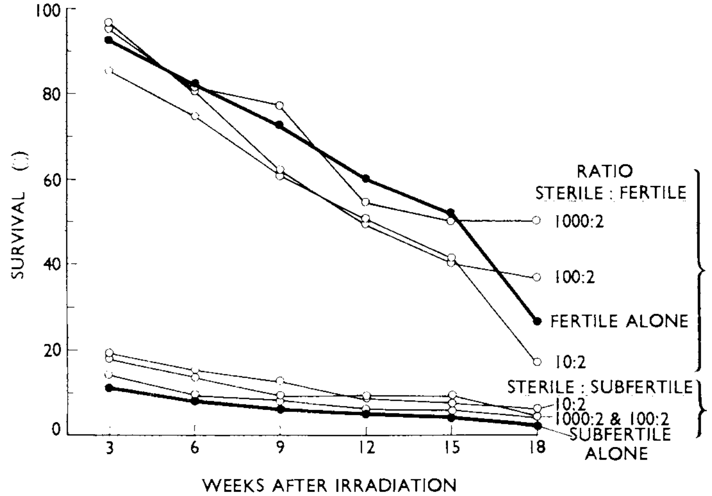


Fig. 4. Survival of fertile (unirradiated) and subfertile (6000 rads) adult grain weevils maintained separately, or in combination with populations of sterile weevils (16,000 rads) in various ratios. (Data for the ratio 10,000 :2 is omitted because of unreliable values obtained with only 2 insects.)

age survival transformed to angles, showed that the longevity of the contaminants, whether fertile or subfertile, was unaffected by the sterile adults, irrespective of the ratio, or numbers comprising the ratio (Fig. 4). The conclusion may thus be drawn that any differences in progeny production between the combined and standard treatments may not be attributable to the effects of population size on mortality.

Fertility

A similar analysis could not be carried out on the data for progeny production (Table 7), since in many treatments no progeny were produced. Instead, progeny production was examined separately for the Standard Treatments and then used as a basis for examining the results obtained for the Combined Treatments.

Standard treatments

In the early part of the experiment (during the first four oviposition periods) the yield of progeny was very much affected by parent density, significant negative correlations ($P < 0{\cdot}1$ per cent) being obtained between log density

and progeny per parent (Fig. 5); with loss of adults through mortality, and with a loss of reproductive integrity with age, the number of progeny per parent was relatively unaffected towards the end (oviposition periods 5 and 6). Progeny of the subfertile adults was much reduced: in contrast with the

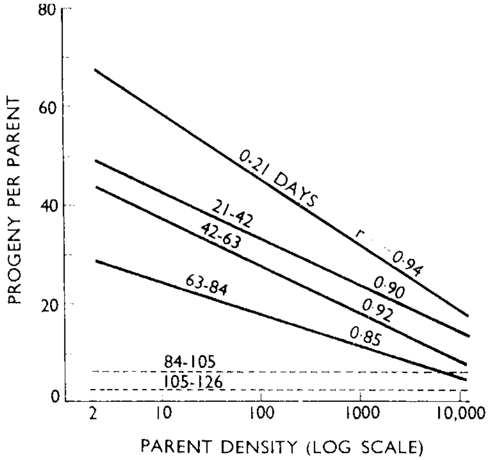


FIG. 5. Relationships between fertility of adult grain weevils (progeny per parent) and population density on 7 kg of grain for 3-weekly oviposition periods.

fertile insects there was a significant positive correlation ($r = 0{\cdot}94$, $P < 0{\cdot}1$ per cent) between the density of subfertile adults and the mean number of progeny per parent in the first three weeks, indicating a greater probability of fertile matings with an increased size of substerile population.

Combined treatments

Using the regression lines derived for Standard Treatments (Fig. 5), progeny produced by the fertile contaminants was expressed as a percentage. The results of these calculations (Fig. 6) may be interpreted as follows:

(1) The ratio of sterile to fertile adults has a marked effect on yield of progeny in all oviposition periods; at a ratio of 10:2, progeny were reduced by up to 50 per cent whilst the sterile population remained alive. The higher the swamping ratio, the greater the depression in reproductive potential; at a ratio of 10,000:2 no progeny were produced whatsoever (Table 7).

(2) The extent to which reproductive integrity is reduced by sterile insects is considerably modified by the numbers of fertile contaminants comprising the ratio. Whilst by a ratio of 10:2 the chance production of a fertile zygote is only 1 in 5, the results show losses in fertility considerably smaller than expected. Surprisingly, too, the results suggest that fertile zygotes are produced more frequently among large populations of contaminants (200 or 2000) than when composed of 2 or 20. The latter result implies that spatial

distribution modifies the theoretical concept. It does not substantiate the suggestion made in the previous experiment that mutual disturbance in large populations may have been responsible for reducing fertility. For a limited period after death of the sterile population (3–6 weeks) the difference in the yield of progeny of small and large numbers of contaminants comprising a

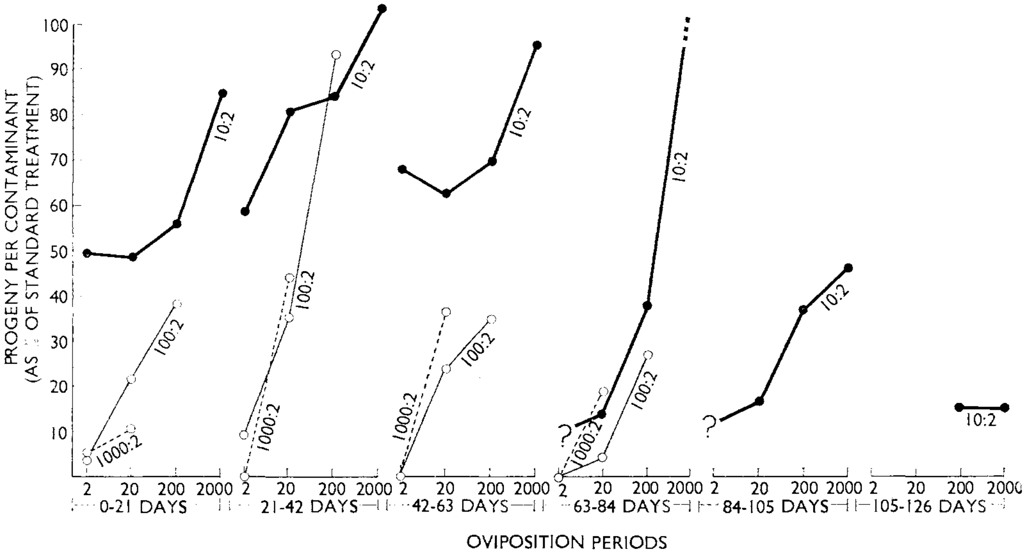


FIG. 6. Reproductive integrity of fertile grain weevils maintained as contaminants to sterile populations in various ratios, composed of different numbers of adults (abscissa). Progeny per contaminant expressed as percentage of fertile adults maintained in the absence of sterile insects. (Zero values were obtained with the ratio of 10,000 :2.)

particular ratio tends to become more marked. A stage is reached, however (in the fifth and sixth oviposition periods), when fertility is equally depressed irrespective of numbers comprising the ratio.

(3) For the first 10 to 12 days of the experiment the ratio of sterile to fertile insects is approximately constant. Thereafter, rapid death of the sterile insects between 14 and 21 days drastically reduces the initial swamping ratios, and at the end of the first oviposition period no sterile insects survive to provide sterile sperm. The effects of sterile insects among the population during the second and subsequent oviposition periods can thus only remain in the form of competitive behaviour of sterile and fertile sperm inseminated whilst the sterile insects were alive. This type of competition must also change in time since only interfertile matings can occur after the first oviposition period.

The results show that the higher the numbers of contaminants comprising a particular ratio, the longer do they maintain reproductive integrity after death of the sterile population. For instance, at the 10:2 ratio, there was

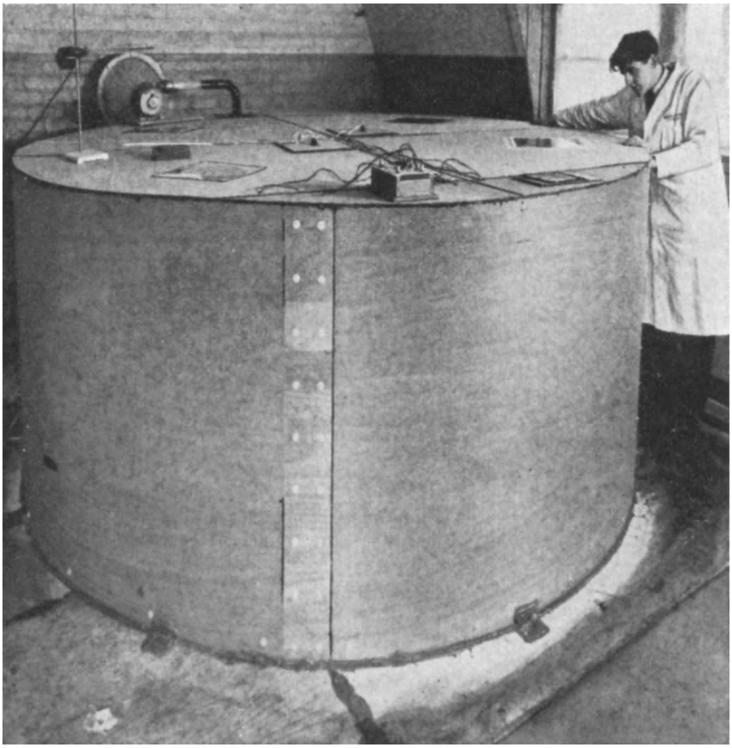


PLATE 1. Experimental bulk storage of 5 tons of wheat to confirm the efficacy of 16,000 rads for complete control of 10 million weevils *(S. granarius)* in all stages of development.

little or no depression of reproductive ability of 2000 contaminants throughout the first four oviposition periods – findings similar to those of the earlier experiment. At the same ratio with 200 contaminants, yield of progeny was depressed to 55 per cent in the first oviposition period, increased to 84 per cent in the second period, and fell to 70 per cent and 40 per cent in the third and fourth. In the fifth and sixth oviposition periods there was a marked decline in yield of progeny, irrespective of the numbers comprising the ratio. The explanation is, probably, that with advancing age, frequency of mating becomes very much reduced, the quantity of fertile sperm within the female is not retained at the same level, and sterile sperm, not completely depleted, is more competitive in quantity. Without dissection and cytological study it is not possible to comment on the comparative behaviour of sterile and fertile sperm within the female. One conclusion is clear, however – that to whatever extent the contaminants are outnumbered, there is no evidence of a gain in the reproductive potential of the contaminants immediately after the sterile population has died. Reproductive integrity never returns to the level of normal fertile adults. On the contrary, there is evidence to suggest that sterile sperm, once inseminated, can remain competitive for a very long time with fertile sperm subsequently introduced.

(4) Because of the extremely low level of residual fertility in adults irradiated at 6000 rads, the effect of sterile populations on subfertile contaminants could not be examined in the same detail. Examination of the data in Table 7 shows that whilst the sterile insects remained alive, a ratio of 10:2 depressed the already low measure of fertility of the subfertile females by a further 70 per cent. At 100:2, progeny were produced only by the highest number of contaminants (200) and then in numbers constituting only 25 per cent of those produced by subfertile adults alone. At a ratio of 1000 :2 and 10,000 :2, reproduction was completely inhibited: these treatments, however, contained not more than 20 subfertile insects, which in these low numbers failed to produce progeny in the standard treatments. When subfertile contaminants remained alive after the sterile population had died, progeny were produced only occasionally at a ratio of 10 :2, and only where large numbers of insects were involved, viz., 10,000 + 2000 and 1000 + 200. This evidence supports the findings obtained with fertile contaminants, namely, that the numbers of insects comprising the swamping ratio modify the resultant level of fertility.

EFFICACY OF THE STERILISING DOSE (16,000 rads) AGAINST A WILD STRAIN IN BULK STORAGE

Samples of grain taken from 5 tons of English wheat (Cappelle), contained in a plywood silo (Plate 1) and infested with a wild strain of *S. granarius*, revealed a population of about one million adults and 8–10 million immature stages. The whole 5 tons was irradiated at an average dose rate of 2750 rads/hr

to give a calculated minimum dose of 16,000 rads. Slight variations in dose distribution, however, (mean 16,500 rads ± 5 per cent) resulted in an actual minimum of 15,700 rads. The grain was irradiated in the Wantage Package Irradiation Plant(14) in kraft paper bags each containing 28 lb. Tests were also carried out, concurrent with the bulk irradiation, to compare the susceptibility of the wild strain with that of the laboratory standard.

Characteristics of the strain

The first comparison was made between adults taken direct from the infested storage and adults of the laboratory strain bred under crowded conditions. Culture environment during weevil development has been shown to modify radiation susceptibility,(15) and a second comparison was therefore made with the wild and laboratory strains reared identically on 7 kg of Manitoba wheat inoculated with 250 adults.

TABLE 8

Radiation susceptibility of the standard and wild strains

Test	Strain	Equation Y (probit) =	LD 50% kill (at 28 days) Log dose ± S.E.	Dose (rads)
1	Standard	$-8{\cdot}987x + 33{\cdot}603$	3·739 ± 0·0026	5483
	Wild	$-8{\cdot}428x + 32{\cdot}026$	3·800 ± 0·0028	6310
2	Standard	$-15{\cdot}354x + 57{\cdot}547$	3·748 ± 0·0036	5598
	Wild	$-14{\cdot}644x + 54{\cdot}969$	3·754 ± 0·0035	5675

TABLE 9

Fertility and adult weight of the standard and wild strains

Test	Strain	Total progeny	Progeny/ parent/day	Mean adult weight (mg)	No. of adults weighed
1	Standard	6508	1·86	2·60	730
	Wild	2396	0·68	2·07	1430
2	Standard	13,605	3·89	2·61	1320
	Wild	6128	1·75	2·24	1300

Results of the first test (Table 8) showed that the wild strain was significantly more resistant to radiation than the standard, but when both strains were reared under identical conditions the difference in susceptibility was no longer significant. Tests on fertility and mean adult weight showed that in

both tests values derived for the wild strain were lower than for the standard (Table 9). Adults of the wild strain were noticeably darker and showed a pronounced death-feigning response when disturbed, quite unlike that of the standard.

Efficacy of the treatment

During irradiation of the bulk grain the silo was thoroughly cleaned and moved to another site to avoid possible contamination by unirradiated insects. It was then filled with the irradiated grain and carefully sealed; thermocouples were installed centrally at three levels. The air space above the grain was ventilated at 17 °–25 °C, no attempt being made to control relative humidity.

The upper surface of the grain was examined periodically over 15 months and no live weevils could be found. At the end of this period the whole bulk of grain was sieved. All weevils found were dead. The grain appeared to be in the same condition as at the time of irradiation. The moisture content of the grain ranged from 12 per cent – 15 per cent, indicating that in the event of incomplete sterilisation immature stages would have been able to develop.[11]

DISCUSSION

In comparing ionising radiations with fumigation for the control of insects in grain,[16] the efficacy of both methods was thought to depend equally on adequate storage facilities, neither process offering any protection against reinfestation. In the same review, a contrast was made between the two processes with respect to population changes after application of the control measures; the reproductive potential of insects surviving sublethal doses of insecticide is in no way affected by the treatment, whereas sublethal doses of ionising radiations reduce the population to a low level, the deleterious effects of irradiation on the germ cells suppressing population increase for many months.

The present studies have shown that 14,500 rads reduces a population of grain weevils to a very low level, which is without increase over a period of 5 to 8 months. The lower doses of 10,000 and 12,000 rads (63 per cent and 75 per cent of 16,000 rads, shown by this study to be completely effective against a massive population of a wild strain in bulk storage) can suppress large weevil populations for up to 4 months. With smaller populations of weevils the control period is extended to more than 6 months. In interpreting this information it is important to assess the magnitude of these experimental populations in relation to those encountered in grain storage practice. Matz[17] states that an infestation level of grains of more than 0·3 per cent is not acceptable for commercial production of flour. The lowest population of insects examined in this study (7500 on 4 cwt of grain) constitutes a 0·1 per cent

level of infestation and the two higher levels were 1 per cent and 10 per cent. Clearly, the two higher population densities examined in this study are considerably more severe than are likely to be encountered in commercial practice.

Treatment of weevils under reduced oxygen is less effective by a factor of 60 than irradiation under atmospheric conditions. Again, the experimental conditions with respect to gas tension, which resulted in temporary asphyxiation of the insects, are unlikely to be paralleled in commercial practice. Despite the severity of these experimental conditions, increase in the size of low infestations was suppressed for as long as 6 months; with no evidence of a population increase after this period, there is good reason to believe that 10,000 and 12,000 rads would be effective for normal storage periods.

Experiments to examine the effect of sterile insects in combating the reproductive potential of insects which gain access after irradiation, showed that in the first, with irradiation under reduced oxygen, low numbers of fertile insects (1 fertile : 500 sterile) produced only $\frac{1}{30}$ of their normal progeny in the first fortnight and only $\frac{1}{6}$ in the subsequent 2 months. As the number of contaminants increased, so the protection afforded by sterile insects was reduced (Table 10). No information is available on the numbers of insects which normally constitute reinfestation; under modern storage management, a reinfestation ratio of 1 adult to 500 in a treated population would probably not be tolerated and might well necessitate repeated fumigation. A ratio of 1 in 50 might be experienced with bagged storage in warm climates.

TABLE 10

Percentage fertility of adult grain weevils contaminating almost sterile populations at different ratios

(Expt. 1: Irradiation at 16,000 rads under reduced oxygen)

Ratio of contaminants	Weeks after irradiation 0–2	2–6	6–10
Fertile 1:500 Almost sterile	3	12	16
Fertile 1:50 Almost sterile	13	70	62
Fertile 1:5 Almost sterile	52	86	77

The second experiment to examine the effect of sterile sperm in preventing reinfestation supported the findings of Table 10. The reduction in fertility of contaminants at 1:500 was not, however, so severe; 10 per cent in the first 3 weeks, and 40 per cent and 50 per cent in the subsequent 6 and 9 weeks (Table 11). This discrepancy must surely be attributed to the survival of a small fraction of the "sterile" insects in the first experiment compared with complete mortality by 3 weeks in the second.

Population size was also found to modify the extent of partial protection, possibly an expression of the physically limited environment. More important, sterile sperm was found competitive within the female for 18 weeks, even though the initial infestation was completely killed soon after irradiation. As weevils treated with the sterilising dose (16,000 rads) die within 3 weeks after treatment, fertile insects which gain access after this short period

TABLE 11

Percentage fertility of adult grain weevils contaminating completely sterile populations at different ratios

(Expt. 2.* Irradiation at 16,000 rads under atmospheric conditions)

Ratio of contaminants	Weeks after irradiation 0–3	3–9	9–18
Fertile 1:500 Completely sterile	10	42	54
Fertile 1:50 Completely sterile	30	47	39
Fertile 1:5 Completely sterile	63	85	64

* Data averaged for different-sized populations and oviposition periods. Treatments involving only 2 contaminants are omitted because of unreliability: the 2 adults (1♂ and 1♀) swamped at 1:5000 gave no progeny.

cannot be inseminated with sterile sperm. The conditions in the present study have their closest parallel in handling operations which involve the placement of irradiated grain in a contaminated silo bin or boat hold. Fumigation of grain *in situ*, in bins or boat holds, may control the resident populations in the bins or boat holds at time of treatment, but the product is in no way protected when handled for transit or storage. If it is desirable to provide an extended measure of protection, the sublethal but almost completely sterilising doses near 10,000 rads might be considered.

Experiments involving the interaction of subfertile with sterile insects showed that the low reproductive potential of subfertile contaminants was further considerably reduced. Treatment with 6000 rads itself lowered fertility to about 0·6 per cent; the results suggest that on Probability alone, treatment with this dose could result in populations of less than about 100 adults producing only the occasional progeny. When adults which receive only 6000 rads constitute up to 20 per cent of a sterilised population, their reproductive potential is further reduced to one-third, viz. to about 0·2 per cent of untreated weevils – equivalent to the whole population having received about 8000 rads.(1) Clearly, in a commercial application a small proportion of the infested product could be treated with as little as 40 per cent of the sterilising dose without loss in efficacy – underdosing, with 10,000 or 12,000 rads, could be tolerated to an even greater proportion of the product. This gives considerable flexibility to the specification for uniformity of dose in plant design.

The test of 16,000 rads against a massive population of weevils of a wild strain gives a considerable measure of assurance that this dose would be highly effective in practice without adding arbitrary increments of dose as safety factors. Any measure of overdosing, however small, and to however limited a proportion of the product, constitutes a loss in energy and adds to the operating cost. These studies have indicated that 16,000 rads is the maximum likely to be required for practical control of weevils. A generous amount of underdosing is considered acceptable.

CONCLUSIONS

1. Irradiation of grain weevils under reduced oxygen with 10,000 rads suppresses populations ranging from 7500–750,000 adults to a very low level for up to 4 months. With 12,000 rads and treatment of 750,000 weevils, increase of the suppressed population is delayed until 5 months; with samples of 7500 and 75,000 adults no population increase can be detected at 6 months. With 1000–1,000,000 adults treated with 14,500 rads, population increase is first observed after periods ranging from 5 to 8 months after irradiation.
2. Irradiation under atmospheric conditions with 10,000 rads suppresses 750,000 adults to 700 (0·1 per cent) 2 months after treatment, increasing to 41,000 (6 per cent) at 7 months. Similar treatment of 75,000 and 7500 adults suppresses population increase until 4 months. With 12,000 rads given under atmospheric conditions, 750,000 adults are reduced to 200 (0·03 per cent) from 2 to 4 months after treatment, increasing to 1300 (0·2 per cent) at 7 months. Twelve thousand rads gives complete control of 7500 adults with no live insects present at 7 months.
3. Residual populations of weevils, 2–4 months after treatment with 10,000 and 12,000 rads, given under reduced oxygen, are 60 times greater than when irradiated in air. Under both conditions of gas tension, residual populations 2–4 months after irradiation are 4 times greater after treatment with 10,000 rads than with 12,000 rads.
4. Irradiation of adults with 16,000 rads under reduced oxygen is sublethal and incompletely sterilising; 26 per cent of adults survive 2 weeks after irradiation, 5 per cent for 6 weeks and 3 per cent for 10 weeks; a sample of 10,000 produced 700 progeny in 10 weeks. All of 10,000 weevils treated with 16,000 rads under atmospheric conditions died within 3 weeks and produced no progeny.
5. Irradiation provides partial protection to treated grain through the insemination of contaminants with sterilised sperm. The reproductive potential of fertile weevils is reduced when added to populations of adults treated with 16,000 rads. With irradiation under reduced oxygen (substerilising and sublethal) the fertility of contaminants at 1:500 is sup-

pressed to 3 per cent in the first fortnight and to 16 per cent in the subsequent 2 months. At ratios of 1:50 and 1:5, fertility is reduced to 13 per cent and 52 per cent, respectively, during the first fortnight, and to 66 per cent and 81 per cent during the following 2 months. With irradiation at 16,000 rads under atmospheric conditions (completely sterile and lethal) fertility of contaminants at 1:500 is reduced to 10 per cent and 48 per cent during the first 3 weeks and subsequent 4 months: the corresponding values for these periods are 30 per cent and 44 per cent respectively, at a ratio of 1:50, and 63 per cent and 75 per cent at a ratio of 1:5. Sterile sperm remains competitive with fertile sperm, subsequently introduced, for a period of at least 18 weeks.

6. The fertility of adults treated with 6000 rads is reduced to 0·6 per cent of unirradiated insects. The reproductive potential of these subfertile weevils is further reduced to 0·2 per cent when combined at a ratio of 1:5 with insects treated at 16,000 rads. Insemination of subfertile insects with sterile sperm allows a considerable measure of underdosing to part of the grain without loss in efficacy. Accordingly, it permits considerable flexibility in specification of dose uniformity in the design of irradiation plant.

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PAPER VII

The Effect of Rearing Medium on the Susceptibility of *Tribolium confusum* Duv. and *Sitophilus granarius* (L.) to Gamma Radiation

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Rate of development of *Tribolium confusum* Duv. and the yield of adults on different milling fractions is influenced by the chemical composition of the cereal medium. Rapidly developing flour beetles are more susceptible to killing by gamma radiation when measured at 21 days; the period before latent radiation damage culminates in death is also shortened. Susceptibility at 28 days is not modified by rearing medium. Rate of development of *Sitophilus granarius* (L.) on different whole cereals and the yield of adults appear not to be directly correlated with chemical composition; they are more probably influenced by physical factors such as grain size. Weevils reared on different grains show differences in susceptibility to gamma radiation at the LD 50 which cannot be correlated with any of the experimental indices considered.

INTRODUCTION

Under conditions of commercial grain storage, the majority of insect pests attack a variety of different cereals; *Tribolium* species infest milled products as well as whole grains and *Sitophilus* is encountered, in varying degree, on all cereals of importance as food to animals and man.

Industrial exploitation of ionising radiations for the control of insect pests of grain is likely to involve treatment at port terminals, principally of wheat, but to a smaller extent of the coarse grains, maize, oats, rye and sorghum. The radiation susceptibility of insects which have developed on different media must therefore be considered for successful application of this process.

Two experiments are here described in which *Tribolium confusum* were reared on seven milling fractions and *Sitophilus granarius* on nine whole cereals. Adults so obtained were tested for susceptibility to gamma radiation from ^{60}Co. In a previous communication,[1] culture environment with particular reference to temperature was found to modify the lethal response of

* This investigation was carried out while the author was at the Wantage Research Laboratory, Isotope Research Division, (A.E.R.E.) Wantage, Berks.

grain weevils to irradiation, but their susceptibility to sterilisation was unchanged. The present work was therefore confined to examining modifications induced by the rearing medium on the dose of radiation required to kill. Whilst there may be no immediate interest in the application of radiation for the treatment of flours, milling fractions provide a ready means of testing the possible influence of food on the radiation susceptibility of *T. confusum*.

METHODS

Facilities for rearing insects were controlled at 27 °C and 70 per cent r.h. Irradiations were carried out at a constant dose rate of 4000 rads/hr, the range of doses being obtained by varying the exposure time. During irradiation the insects were contained in ventilated polythene tubes on small quantities of the foods being investigated.

The effect of different milling fractions on the susceptibility of *T. confusum* to gamma radiation

In the first experiment cultures of *T. confusum* were reared on 7 milling fractions, including wholemeal, milled from a "bakers" grist of 15 per cent English wheat (Cappelle), 10 per cent Plate Wheat, 50 per cent Manitoba No. 2 and 25 per cent Manitoba No. 3 wheats.

The fractions ranged in carbohydrate content (Table 1) from 50 to 70 per cent and in protein from 13 to 21 per cent; there were minor variations in oil fibre and ash, but in "J tails" these constituents were considerably more predominant. There were noticeable variations in particle size of the fractions but these were not measured; all fractions were equilibrated with a relative humidity of 70 per cent at 27 °C before use for culture.

TABLE 1

Chemical composition of milling fractions (%) arranged in order of minimum development time for culture of T. confusum *(see Table 2)*

Fraction (milling designation)	Carbo-hydrate	Protein	Oil	Fibre	Ash	Mois-ture
"G" dunst.	68·5	14·3	1·8	0·5	1·0	13·9
4th break scalpings	60·8	19·0	2·7	1·5	1·6	14·5
Grist (wholemeal)	68·9	15·0	2·1	1·7	1·6	10·9
"J" tails	51·8	21·4	5·9	5·8	4·8	10·4
"J" flour	71·0	14·1	1·7	0·4	0·6	12·3
"G" flour	71·4	13·5	1·3	0·2	0·4	13·4
"B_2" flour	72·2	13·2	1·1	0·2	0·4	13·1

About 1500 eggs of *T. confusum* were first placed on 1 kg of each fraction and incubated in a half gallon culture jar to assess its merit as a culture medium. The numbers of adults which developed, expressed as an accumulating total, are shown in Fig. 1. From the first three fractions, (Table 2), adults emerged at a similar rate and about 1450 were obtained; fewer adults, about 1000–1200, were obtained from the remaining four fractions and the rate of emergence was retarded, particularly on flours "G" and "B_2". Using this data, the incubation period for 90 per cent emergence – an index of rate of development, was found to range from 40 to 52 days. The adults varied only slightly in weight from 1·9 to 2·2 mg (Table 2).

TABLE 2

Development characteristics of adult T. confusum *reared on different milling fractions, together with their susceptibility to gamma radiation*

Fraction (milling designation)	Development			Susceptibility		
	Total yield of adults	Incubation period (days) for 90%	Adult wt. (mg)	LD 50 21 days (rads)	LD 50 27 days (rads)	LT 50* (days)
"G" dunst	1469	40	2·2	9110	8540	14·5
4th break scalpings	1439	40	2·1	9010	8550	14·9
Grist (wholemeal)	1417	41	2·1	8970	8470	14·6
"J" tails	1133	41	2·1	9830	8860	15·9
"J" flour	1032	42	2·2	9770	8750	16·4
"G" flour	1212	47	1·9	9970	8840	17·2
"B_2" flour	1042	52	2·1	10,810	—	18·0

* LT 50 = the time in days for 50 per cent kill averaged for six doses ranging from 11,200–25,000 rads (see Fig. 2).

To test adults reared on the different fractions for susceptibility to gamma radiation, a second series of cultures was set up using about 3500 eggs on each, to provide sufficient numbers of adults for treatment within a narrow age range, 8–14 days old. These were irradiated in batches of 100 at 15 dose levels ranging from 1000–25,000 rads; they were then maintained on 100 g of the appropriate milling fraction and examined for mortality at 3-day intervals.

The rate of death of adults reared on the seven fractions is averaged in Fig. 2 for the dose range 11,200–25,000 rads, in which complete kill was obtained. Delayed response to irradiation as measured by the time for 50 per cent kill varied from just under 15 days to 18 days (Table 2). The LD 50, calculated from fitted regressions of probit mortality on log dose 21 days after irradiation, ranged from 8970 rads for beetles bred on wholemeal to 10,810 rads for those bred on B_2 flour (Table 2). Calculation of the variance

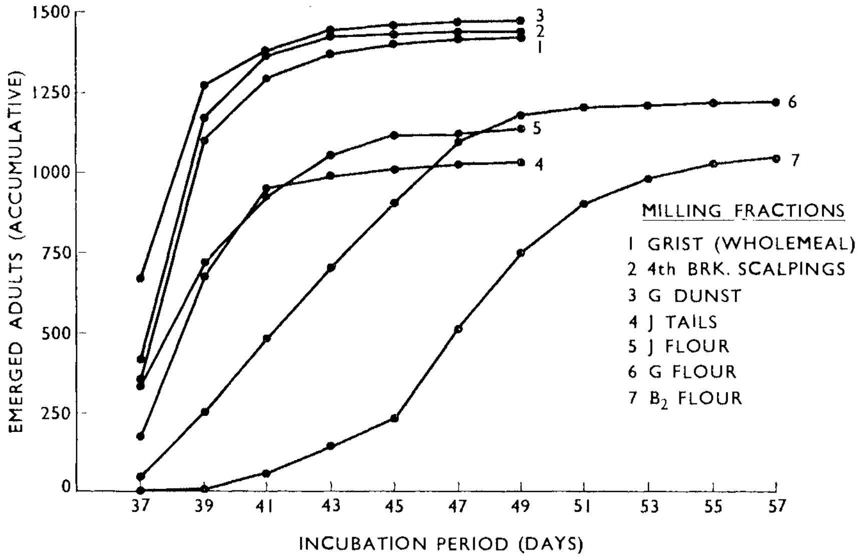


FIG. 1. Yield and rate of emergence of adult *T. confusum* reared on various milling fractions.

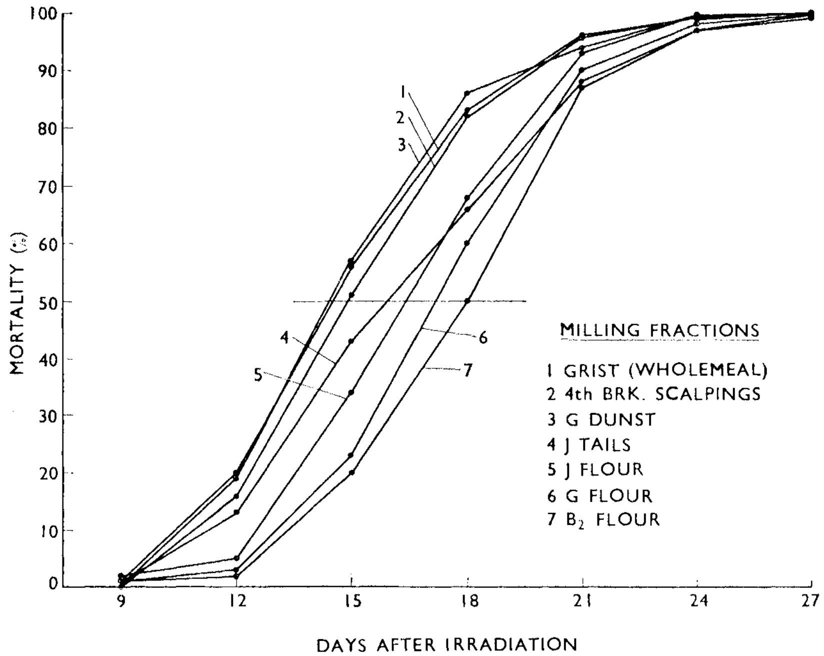


FIG. 2. Mean rate of mortality of irradiated adult *T. confusum* (dose range, 11,200 to 25,000 rads) reared on various milling fractions.

and confidence limits around the fitted lines showed highly significant differences in LD 50 (P either < 1 per cent or 0·1 per cent) between the more resistant adults reared on flours G and B_2 and the more susceptible beetles reared on G dunst, scalpings and grist. Table 2 also shows that the LT 50 and LD 50 at 21 days were closely correlated. The LD 50 at 27 days showed a

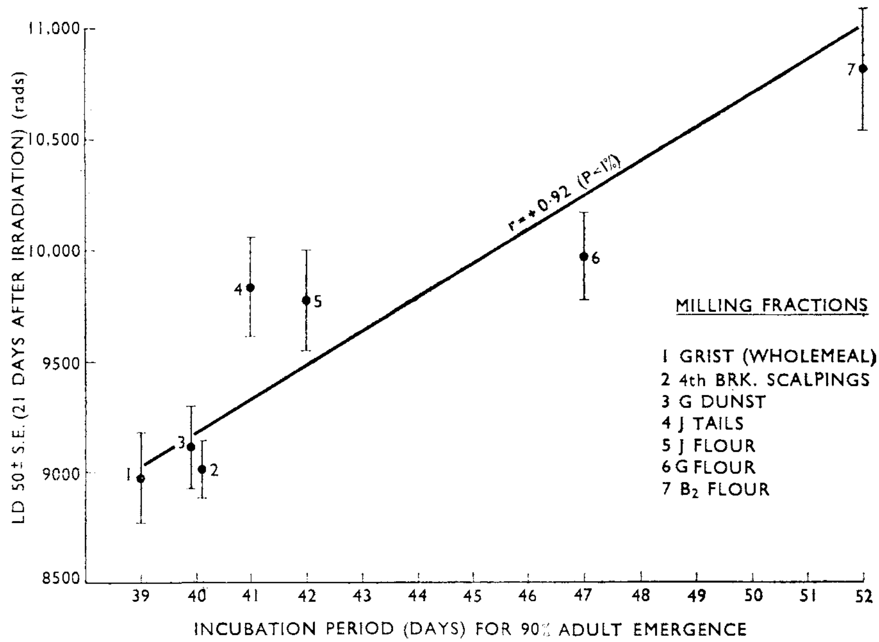


FIG. 3. Relationship between susceptibility of adult *T. confusum* to gamma radiation and rate of development on various milling fractions.

much narrower range in susceptibility from 8470 to 8870 rads, indicating little influence of rearing medium on susceptibility when the full effects of irradiation had taken place (Fig. 2).

Further examination of the data, with the exclusion of "J tails" (Tables 1 and 2) showed a positive curvilinear relationship between increasing LD 50 at 21 days and the carbohydrate content of the fractions, and negative relationships between LD 50 and protein, oil, fibre and ash. Of greater interest, however, was the close correlation ($r = +0{\cdot}92$, $P < 1$ per cent) between susceptibility to irradiation (LD 50, 21 days) and the rate of development (90 per cent emergence) on all seven fractions (Fig. 3). The small differences in LD 50 at 27 days indicates that the changes in susceptibility induced by the milling fractions are transient and the ultimate response to irradiation is unaffected.

The effect of different cereal grains on the susceptibility of *S. granarius* to gamma radiation

In the second experiment, cultures of *S. granarius* were reared on nine whole cereals, four of them wheats of different origin (Table 3). Chemical analyses showed a range in carbohydrate content from 63 to 72·5 per cent and in protein from 8·2 to 15 per cent. There were minor variations in oil, fibre and ash,

TABLE 3

Chemical composition of cereal grains (%) arranged in order of minimum development time for culture of S. granarius *(see Table 4)*

Cereal grain	Carbo-hydrate	Protein	Oil	Fibre	Ash	Moisture
English wheat	69·7	11·2	1·3	2·3	1·9	13·7
Manitoba wheat	66·2	14·8	1·4	2·9	1·6	13·2
U.S.A. wheat	66·7	15·0	1·4	2·4	1·7	13·0
Barley*	—	—	—	—	—	—
Rye	72·5	9·1	1·3	2·0	2·1	13·1
Australian wheat	70·8	10·9	1·3	2·5	1·4	13·2
Sorghum	72·0	8·9	2·6	2·4	1·4	12·8
Oats	63·0	8·2	5·5	9·0	2·1	12·0
Yellow maize	70·9	8·5	3·8	2·4	1·3	13·3

* Insufficient quantity available for analysis.

with the exception of sorghum, oats, and maize which were high in oil, oats alone having a high content of fibre. In general the grain size varied between 20 and 30 grains/g except for rye, the smallest, which averaged 36 grains/g and maize, the largest, which averaged 4 grains/g. All were equilibrated with a relative humidity of 70 per cent at 27 °C before use for culture.

To assess the value of each cereal as a rearing medium for weevils, batches of 200 adults were placed to oviposit for 4 weeks on 7500 cm^3 of each grain in two-ply kraft paper sacks.* The adult progeny were removed weekly and held in further sacks of grain for irradiation when 7–28 days old.

* The merit of using similar volumes of each cereal was compared with that of using similar numbers of grains, or equal weights of grain. Total mass is relatively unimportant as each grain usually contains only 1 developing insect: maize is the principal exception with 3 larvae and sometimes more occasionally able to develop in one grain. Equal volumes of grain ensured that each sack was filled to the same extent, allowing similar conditions for ventilation of respiratory gases, loss of heat and moisture. Variation in adult density of 100 to 1000 weevils ovipositing for 28 days in paper sacks has been found not to influence the radiation susceptibility of their progeny.

The yield of adults in the first 4 weeks of emergence varied widely; 12,000 to 22,000 were obtained from sorghum, Australian and Manitoba wheats, rye, American and English wheats in ascending order (Table 4). Only 3500 and 8500 were obtained from maize and barley, respectively, and less than 1000 from oats. Thickness of the oat husks and the hard testa of the maize

TABLE 4

Development characteristics and radiation susceptibility of adult S. granarius *reared on different cereal grains*

Cereal grain	Development			Susceptibility	
	Total yield of adults	Incubation period (days) for 90%	Adult wt. (mg)	LD 50 (rads)* at 21 days F_1	F_3
English wheat	22,480	41·5	2·8	6,410	6,510
Manitoba wheat	16,290	41·5	2·7	5,410	5,780
U.S.A. wheat	20,280	41·9	2·6	5,920	6,000
Barley	8,530	42·4	2·9	6,180	6,390
Rye	18,060	43·6	2·0	6,080	6,430
Australian wheat	15,120	43·6	2·4	5,720	6,060
Sorghum	12,190	44·2	1·8	5,200	6,100
Oats	790	49·0	1·9	6,060	—
Yellow maize	3,510	49·2	2·9	5,870	5,850

* Susceptibility after rearing for 1 and 3 generations, respectively on the different cereal grains.

may have reduced the ability of females to oviposit. Yield of adults was not correlated with speed of development (incubation period for 90 per cent emergence) which varied from 41 to 49 days. Neither was it correlated with size of progeny. There was a tendency, however, for larger weevils to be obtained with more rapid development, but with notable exceptions; maize, the largest grain, produced the heaviest weevils and the small oats and sorghum produced the lightest.

Tests for radiation susceptibility were made with batches of 100 weevils at each of 12 doses (1000–10,000 rads) with further tests carried out after rearing on each cereal for three generations.* The irradiated insects were held on 1 kg of grain in jars. Calculated LD 50's at 21 days (Table 4) for the first generation ranged from 5200 to 6400 rads. Variances about the LD 50 were sufficiently small to demonstrate differences in susceptibility at the 1 per cent or 0·1 per cent level of probability (Table 5). No correlation could be found between susceptibility and chemical composition of the grains, or between

* The number of weevils which could be reared on oats was insufficient to test the third generation for radiation susceptibility.

susceptibility, speed of development, or size of adults. The LD 50's of the third generation were generally a little higher than the first but only statistically so for weevils reared on sorghum ($P < 1$ per cent), Manitoba wheat and rye ($P < 5$ per cent).

TABLE 5

Statistical differences between LD 50's of adult S. granarius *reared on different cereals for one and three generations before subjection to gamma radiation*

Probability < 0·1%	Probability < 1%
First Generation	
English – Manitoba	English – Australian
English – Sorghum	Manitoba – Oats
Rye – Sorghum	Manitoba – Rye
	U.S.A. – Sorghum
	Oats – Sorghum
Third Generation	
English – Manitoba	English – Australian
English – Maize	English – U.S.A.
Barley – Manitoba	Barley – Maize
Rye – Manitoba	Rye – Maize
	Rye – U.S.A.

DISCUSSION

Among physical factors which have been tested for their effect on the susceptibility of insects to gamma radiation, dose rate,[2] temperature,[3] dose fractionation[4] and oxygen tension[5] have considerable modifying influence. The effects of diet and the nutritional content of infested foods have not previously been examined. The present work has shown that rate of death of the confused flour beetle after treatment with gamma radiation is retarded when the rearing medium causes a slow rate of development; these are media with a relatively high content of carbohydrate and a low content of protein, oil, fibre and ash. Nevertheless rearing medium was not found to influence mortality 4 weeks after irradiation, at which time the mortality response is complete. Since rate of development is correlated with yield of adults, it is possible that the differences observed in response to irradiation may be attributed to slight differences in culture temperature.[1]

The susceptibility of grain weevils to gamma radiation could not be correlated with rate of development, yield of adults, or chemical composition of the wheats. These cultures were held in large paper sacks in which it would not have been possible for significant rises of temperature to have occurred

during rearing. There were, however, marked differences in the LD 50's of weevils reared on different whole cereals for which no explanation can be offered.

A possible explanation of the failure to demonstrate an effect of diet on the susceptibility of *T. confusum* (4 weeks after irradiation) may be found in the association of these beetles with beneficial microflora,[6] which may compensate for dietary deficiencies. The responses of *S. granarius* may be attributable to an interaction of factors, such as, nutritional content, rate of development, size of grain, hardness, moisture content, weight of adult. It is clear, however, that the susceptibility of both species to gamma radiation is not sufficiently modified by rearing medium to influence the efficacy of radiation disinfestation in commercial practice.

CONCLUSIONS

1. The rate of development of *T. confusum* on various milling fractions is affected by chemical composition. Rapidly developing beetles are more susceptible to the lethal effects of gamma radiation 21 days after treatment but at 27 days no differences can be detected.
2. Development of *S. granarius* on various whole cereal grains cannot be correlated with chemical composition; other factors such as grain size, hardness and moisture content may be implicated.
3. The efficacy of radiation disinfestation of grain is unlikely to be reduced by the type of cereal infested.

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PAPER VIII

Susceptibility of the Confused Flour Beetle, *Tribolium confusum* Duv., and the Rust-red Flour Beetle, *Tribolium castaneum* (Herbst.), to Gamma Radiation

E. J. BANHAM and L. J. CROOK

The minimum effective dose (16,000 rads) for radiation disinfestation of grain has been derived from studies of the radiation susceptibility of the grain and rice weevils, *Sitophilus granarius* (L.) and *Sitophilus zeamais* Mots. The present study was carried out to compare the susceptibilities of the developmental stages of the two flour beetles *Tribolium confusum* Duv. and *Tribolium castaneum* (Herbst.) and to establish the efficacy of 16,000 rads for their control: this dose is completely sterilising but allows 10 per cent of adults of *T. castaneum* to survive.

INTRODUCTION

The need for lower levels of insect contamination in harvested foodstuffs and the hazards to health from residues, which result from the application of certain chemical insecticides in increasing amounts, has led to research into the potentialities of ionising radiations as an additional method of insect control. As part of this experimental programme, the studies reported here were carried out to examine the radiation susceptibilities of all developmental stages of the two species of flour beetle, *T. confusum* and *T. castaneum* with particular reference to the degree of control afforded by 16,000 rads, the minimum effective dose for the complete sterilisation of the grain and rice weevils.[1]

T. castaneum is a major pest in the tropics of a wide range of commodities, including grain, cereal products, oil seeds and nuts. It cannot survive in the U.K. except in heated warehouses, but is frequently found infesting flour mills, which have temperatures higher than are normally encountered in storage. It is one of the most common species occurring on imported food-

stuffs. *T. confusum* is a cosmopolitan species, of secondary importance in grain, the initial infestation frequently arising by cross-infestation from other foodstuffs in transit or storage.[(2)]

EXPERIMENTAL*

Using laboratory strains of the two species, immature stages for irradiation were obtained by incubating batches of 50 eggs in ventilated polythene tubes containing 3 g of wholemeal, milled from Manitoba No. 2 wheat. Samples of

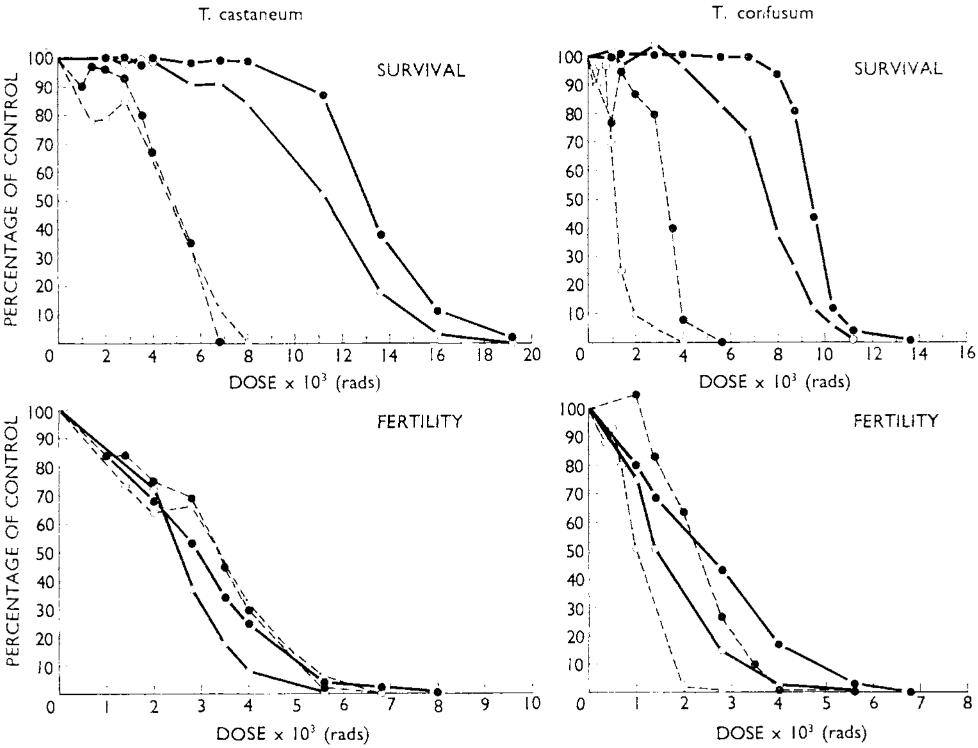


FIG. 1. Survival of the developmental stages of *T. confusum* and *T. castaneum* after the delayed response to irradiation (see Table 2) and total progeny produced in 8 weeks.

○ broken lines ... Eggs
● broken lines ... Larvae
○ continuous lines ... Pupae
● continuous lines ... Adults

* Details of experimental technique, together with biological data for the two species, are given in U.K.A.E.A. Research Reports AERE-R 3888 and AERE-R 3889.

T. confusum were reared at 30°C and 60 per cent r.h. and those of *T. castaneum* at 70 per cent. Eggs and larvae of both species were 2–3 and 14–15 days old, respectively, at irradiation; pupae of *T. confusum* were aged 30–31 days and those of *T. castaneum* 26–27 days. Adults, 0–14 days old, in batches of 50, were similarly contained on 3 g of flour at irradiation. All treatments were carried out in a ^{60}Co source[3] at 4000 rads/hr, using a range of doses (Fig. 1), the samples rotated during irradiation to ensure uniformity of dose ($\pm$2 per cent) Temperature during irradiation was controlled at 30°C and the samples, 10 of immature stages at each dose, and 5 of adults, were at all times contained in insulated flasks whilst in transit between the culture room and the irradiation cell. After irradiation, immature stages were incubated in their tubes until pupation, when pairs of samples were placed together on 100 g of wholemeal in jars. The samples of adults were placed separately on 100 g of flour, immediately after irradiation.

The numbers of adults which emerged from irradiated immature stages were recorded 2 weeks after adults first began to appear, and survivors were then transferred to fresh flour at weekly intervals for 2 months. The irradiated adults were transferred 1 week after treatment and handled in the same way; this allowed valid comparison between stages for survival and progeny production since the samples at transfer were then all of the same age.

RESULTS

The effects of gamma radiation on development to adult

The numbers of adults obtained from irradiated immature stages were calculated as a percentage of the number at risk; this number, for larvae and pupae, was determined from a batch of samples examined at the time of irradiation. The data was then corrected for natural mortality[4] and examined by Probit analysis.

T. confusum

Eggs and larvae of *T. confusum* required, respectively, 1200 and 3400 rads for a 50 per cent reduction, and 4500 and 5300 rads for 99·9 per cent reduction in the numbers of emerging adults (Table 1). In common with the observations on grain weevils[1] emergence was delayed with increasing dose. The emergence of adults from irradiated pupae was in no way affected by irradiation within the dose range investigated.

T. castaneum

The doses required for 50 per cent and 99·9 per cent reduction in adult emergence were, respectively, 5000 and 11,300 rads for eggs and 4900 and 12,600 rads for larvae (Table 1). Emergence was delayed from irradiated

larvae but only at doses above 4000 rads. The number of adults obtained from pupae treated at the highest dose (19,200 rads) was reduced by only 4 per cent.

TABLE 1

Doses for 50 per cent and 99·9 per cent reduction in adults developed from irradiated eggs and larvae of Tribolium confusum *and* T. castaneum. *(Doses for* Sitophilus granarius[5] *are shown for comparison 16,500 rep ≡ 16,000 rads)*

Species	Stage	Dose for 50% reduction		Dose for 99·9% reduction	
		Log dose ± St. Error	Dose (rads)	Log dose ± St. Error	Dose (rads)
T. confusum	Eggs	3·075 ± 0·027	1190	3·654 ± 0·080	4510
	Larvae	3·528 ± 0·010	3370	3·725 ± 0·046	5310
T. castaneum	Eggs	3·699 ± 0·016	5000	4·051 ± 0·050	11,260
	Larvae	3·689 ± 0·026	4890	4·102 ± 0·097	12,650
S. granarius	Eggs		1270 rep		6060 rep
	Larvae		3120 rep		4730 rep

The effect of gamma radiation on survival

Again using Probit analysis, estimates were obtained of the doses required for 50 per cent and 99·9 per cent kill of developmental stages of the two species.

T. confusum

Similarity in the doses for 50 per cent and 99·9 per cent reduction in emergence and survival at 28 days (Tables 1 and 2) indicates very little mortality of adults after emergence, with irradiation as eggs and larvae. The effect of irradiation was essentially one of preventing development to maturity. Adults derived from irradiated pupae died within 28 days of emergence, and irradiated adults died 2–3 weeks after treatment; those surviving remained alive until the end of the experiment.

The dose required to kill 50 per cent of the four stages of *T. confusum* increased from 1200 rads for eggs to 3300 for larvae, 7500 for pupae and 9400 for adults. The corresponding values for 99·9 per cent kill were 4400, 5200, 14,500 and 12,800 rads. The threshold doses for mortality of pupae and adults were approximately 4000 and 7000 rads, respectively. Values for survival, higher than the control, were obtained with pupae irradiated at doses up to 2800 rads and with adults up to 6800 rads, indicating a possible response of stimulation at sub-threshold doses.

TABLE 2

Doses for 50 per cent and 99·9 per cent kill of developmental stages of T. confusum *and* T. castaneum *after the delayed response to irradiation. (Doses for* S. granarius[5] *are shown for comparison; in this species the period of assessment dates from irradiation of all stages)*

Species	Stage (Age in days)	Days from irradiation (adult) or emergence (of immature stages)	Dose for 50% reduction		Dose for 99·9% reduction	
			Log dose ± St. Error	Dose (rads)	Log dose ± St. Error	Dose (rads)
T. confusum	Eggs (2–3)	28	3·068 ± 0·025	1170	3·641 ± 0·073	4380
	Larvae (14–15)	28	3·525 ± 0·012	3350	3·717 ± 0·051	5210
	Pupae (30–31)	28	3·874 ± 0·006	7490	4·161 ± 0·025	14,500
	Adults (0–14)	28	3·972 ± 0·002	9370	4·107 ± 0·016	12,800
T. castaneum	Eggs (2–3)	35	3·704 ± 0·015	5050	4·036 ± 0·057	10,860
	Larvae (14–15)	35	3·668 ± 0·008	4660	4·020 ± 0·072	10,480
	Pupae (26–27)	35	4·020 ± 0·008	10,460	4·411 ± 0·046	25,760
	Adults (0–15)	21	4·120 ± 0·004	13,190	4·332 ± 0·012	21,500
S. granarius	Eggs (0–4)	60		1180 rep		4280 rep
	Larvae (7–11)	60		3030 rep		4350 rep
	Pupae (28–32)	60		4860 rep		8810 rep
	Adults (7–11)	60		4390 rep		9200 rep

T. castaneum

As with the former species, similarity in the doses for 50 per cent reduction in emergence and survival (Tables 1 and 2) with irradiated eggs shows that radiation mortality occurred before adult emergence. With larvae, a drop in the LD 50 of 200 rads and in the LD 99·9 of 2000 rads, indicates some mortality attributable to irradiation after adult emergence. With pupae this response was even more marked; pupae gave rise to adults at all doses, but very few survived more than 2 weeks in the dose range 13,600 to 19,200 rads. Again in common with *T. confusum*, irradiated adults died 2 to 3 weeks after treatment, any survivors then living as well as the controls.

The doses for 50 per cent and 99·9 per cent kill of eggs and larvae were similar (LD 50; 5000 and 4700 rads: LD 99·9; 10,900 and 10,500 rads, respectively – Table 2), followed by a two-fold increase in resistance with incubation to the pupal stage (LD 50; 10,500: LD 99·9; 25,800 rads). The dose for 50 per cent kill of adults (13,200 rads) was significantly higher ($P < 0{\cdot}1$ per cent), but because of differences in the slopes of the regression lines the LD 99·9 was slightly, but not statistically, lower. Pupae and adults irradiated at doses up to 4000 and 8000 rads respectively both gave values for survival exceeding the controls.

The effects of gamma radiation on fertility

Two methods were used to compare the susceptibility of the two species to radiation sterilisation; reduction in total progeny produced over 8 weeks and changes in the mean number of offspring per live parent during each of the 8 weeks. In both instances the data were corrected for variations in the density of parents to that of 50 adults/100 g of flour.* Observations spanning the doses for 50 per cent and 99·9 per cent reduction in progeny (SD 50 and SD 99·9) were analysed by treating the progeny/dose relationship as an exponential function: a more reliable estimate of the SD 50 for *T. confusum* was obtained by Probit analysis.

T. confusum

The estimated doses for 50 per cent reduction in the yield of progeny over the experimental period increased from 900 rads for eggs to 2100 for larvae, falling to 1400 for pupae and increasing again to 2000 rads for adults. The corresponding values for 99·9 per cent reduction were 3400, 6400, 4700 and 7100 rads (Table 3).

Examining first the weekly changes in progeny per parent of unirradiated insects, an increase was obtained with increasing age of adults up to the third week (mean for all stages 27·4 increasing to 32·2) followed by a decrease

* Correction factors were derived from studies of the yield of progeny obtained with different concentrations of adults on 100 g of flour: see U.K.A.E.A. research reports AERE-R 3888 and 3889.

from the fourth to the eighth weeks (27·0 decreasing to 25·5). When the mean progeny per parent per week is averaged for the first and second months of the experiment, the loss in fertility of insects irradiated as eggs and larvae is

TABLE 3

Doses for 50 per cent and 99·9 per cent reduction in progeny of T. confusum *and* T. castaneum *for 8 weeks' oviposition. (Doses for* S. granarius[5] *for one month's oviposition are shown for comparison)*

Species	Stage	Dose for 50% reduction		Dose for 99·9% reduction
		Log dose ± St. Error	Dose (rads)	Dose ± St. Error (rads)
T. confusum	Eggs	2·938 ± 0·012	870	3390 ± 137
	Larvae	3·324 ± 0·005	2110	6420 ± 544
	Pupae	3·153 ± 0·004	1420	4710 ± 526
	Adults	3·292 ± 0·013	1960	7110 ± 823
T. castaneum	Eggs	3470 ± 625		8650 ± 1470
	Larvae	3300 ± 515		8150 ± 1220
	Pupae	2610 ± 250		6190 ± 519
	Adults	3170 ± 136		8590 ± 292
S. granarius	Eggs	1080 rep		2350 rep
	Larvae	2350 rep		4210 rep
	Pupae	1520 rep		4700 rep
	Adults	1410 rep		8680 rep

greater than in controls (Table 4). In pupae and adults, the yield of progeny relative to the controls is greater in the second month than in the first, at least at low doses, indicating recovery in reproductive integrity. No redevelopment of fertility occurred above 9500 rads.

T. castaneum

The very similar response of all stages to radiation sterilisation contrasts markedly with the data for survival. The relationships between progeny production and dose show no significant difference between stages (Table 3) in the SD 50 (2600–3500 rads) or SD 99·9 (6200–8600 rads).

Treatment of the data for progeny per parent per week, in the same manner as described for *T. confusum,* also shows a higher level of fertility of untreated insects in the first month relative to the second (Table 5). *T. castaneum* irradiated in the three stages, eggs, larvae and pupae, all exhibited a lower level of fertility in the second month than the first, relative to the controls; irradiated adults, however, as in *T. confusum,* produced more progeny per parent in the second month than the first. Again there was no redevelopment of fertility in adults treated at high doses – above 13,600 rads.

TABLE 4

Fertility of T. confusum, *expressed as a percentage of the progeny per parent per week of the control (shown) for the first and second months of oviposition. Dashes indicate total death of parents*

Dose (rads)	Eggs		Larvae		Pupae		Adults	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
	Months							
375	100	90						
500	100	94						
700	85	77						
1000	71	70	105	106	71	79	77	83
1400	56	50	89	85	49	48	65	67
2000	22	22	71	62				
2800	7	12	38	31	12	14	40	46
3500			28	21				
4000	0	0	13	13	1	3	14	21
5600			—	—	<1	<1	1	4
6800			—	—	0	0	<1	<1
8000					0	0	<1	<1
8700					0	0	<1	<1
9500					0	0	0	0
10,300					0	0	0	0
11,200					0	0	0	0
13,600							0	0
control	27·5	28·8	28·0	23·9	31·2	24·9	28·8	28·4

DISCUSSION

These experiments have defined the susceptibilities of the four developmental stages of *T. confusum* and *T. castaneum* with regard to development to adult, survival and fertility under carefully controlled experimental conditions. Comparison of the present results with previous studies on mortality,[6,7,8,9] rate of death and sterility[6,9,10] indicates several disparities, which can be attributed largely to variations in experimental method. Work at this laboratory has shown that the temperature before, during and after irradiation,[11] dose rate[12] and culture conditions[13] can each affect the expression of radiation damage. Adequate description of technique is often lacking in susceptibility studies and makes comparisons impossible.

Comparison of the susceptibility of the two species (Table 6) with that of the grain weevil *S. granarius*[5] has been given in Tables 1, 2 and 3. Clearly *T. castaneum* is much more resistant to radiation than either *T. confusum* or *S. granarius* when treated as eggs; the difference between the three species when

TABLE 5

Fertility of T. castaneum, *expressed as a percentage of the progeny per parent per week of the control (shown) for the first and second months of oviposition. Dashes indicate total death of parents*

Dose (rads)	Eggs		Larvae		Pupae		Adults	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
	Months							
1000			100	101				
1400	95	90	89	88				
2000	84	76	82	82	60	52	67	75
2800	77	78	83	74	34	26	46	60
3500			72	61	20	14	27	42
4000	56	42	61	55	8	7	19	33
4800	43	28						
5600	22	13	8	8	<1	<1	2	4
6800	5	2	0	0	<1	<1	<1	2
8000	0	0			0	0	<1	<1
11,200					0	0	<1	<1
13,600					0	0	0	0
16,000					0	0	0	0
19,200					—	—	0	0
control	22·3	21·0	24·2	23·6	23·5	21·0	27·0	23·5

TABLE 6

Ratios of resistance of the developmental stages of T. confusum *and* T. castaneum *to killing and sterilisation by irradiation*

	Stage	Ratio of resistance to radiation: Mortality	Ratio of resistance to radiation: Sterility
		T. confusum : T. castaneum	*T. confusum : T. castaneum*
Dose for 50% reduction	Eggs	1:4·21	1:3·89
	Larvae	1:1·42	1:1·57
	Pupae	1:1·40	1:1·86
	Adults	1:1·40	1:1·60
Dose for 99·9% reduction	Eggs	1:2·48	1:2·56
	Larvae	1:2·02	1:1·28
	Pupae	1:1·78	1:1·32
	Adults	1:1·68	1:1·21

irradiated as larvae is relatively slight. Similarity in the response of eggs and larvae of *T. castaneum* compared with the marked difference between these stages of *T. confusum* is probably because the eggs of the former species were irradiated at a more advanced embryological age. Sudden differences in susceptibility have been found in developing eggs of *S. granarius* and *S. zea-mais*.[1] Differences in susceptibility of pupae and adults of the two related species are also not marked, the ratio of susceptibility (LD 50) being similar to that for larvae, namely 1 : 1·4. The ratio between the species in the dose for 99·9 per cent kill falls from 1:2·5 for eggs to 1 : 1·7 for adults. *T. castaneum* is therefore the most resistant of the three species; the flour beetles are considerably more resistant to killing by irradiation in the pupal and adult stages than *S. granarius.*

At low doses, irradiated pupae and adults of both species of *Tribolium* showed a higher level of survival than controls; at no time, however, was there a similar response by irradiated eggs and larvae. It has been shown by Davey[14] and Cork[15] that X-rays and gamma radiation prolong the life-span of adult *T. confusum* at doses of 3000 rads and below. The stimulatory effects obtained with pupae and adults may be explained by a process of repair leading to increased vigour (Cork, *loc. cit.*[15]), or to an increase in longevity through reduced fecundity; irradiation has been shown to reduce the number of eggs laid by *T. confusum* and *T. castaneum,*[10] which may result in increased longevity of the female.

Comparison of the sterilising effects of radiation on the three species (Tables 3 and 6) demonstrates that eggs of *T. castaneum* are markedly more resistant than either *T. confusum* or *S. granarius,* again possibly for the reasons discussed earlier. Larvae, pupae and adults also require slightly higher doses for 50 per cent sterility. All stages of *T. castaneum* are of similar susceptibility and at the limiting level of fertility (SD 99·9) adults of all three species are also of similar tolerance.

There is some evidence that at substerilising doses, the fertility of adults of both species of *Tribolium* may be recovered after irradiation. The increase in progeny per parent relative to the controls (Tables 4 and 5) may reflect a delay in acquiring maximum fertility through the destruction of gametes. It might also be attributable to a delay in gamete development caused by radiation-induced lethargy,[16] and a time lapse before normal feeding behaviour, mating or oviposition is regained. Park *et al.*[10] have studied the reproductive integrity of irradiated adults of *T. confusum* and *T. castaneum* throughout the full egg-laying cycle (32 and 19 weeks, respectively). Although production of progeny by controls reached a peak at the end of the first month, that of irradiated adults was maximal 2 months after treatment or even later, but complete fertility was never regained. This response by irradiated immature stages is likely to be less marked; adults after treatment as larvae survive well after eclosion, the period of lethargy having passed before egg-laying commences. The germ cells, which are undifferentiated in immature

stages, are also likely to be of equal susceptibility, all as germ cell primordia.

From the viewpoint of control of these species by ionising radiations, it is important to note that there is no redevelopment of fertility at doses approaching the level for commercial application.[1] At the recommended dose, 16,000 rads, effective in controlling large populations of *S. granarius*, there is no likelihood of fertility in *T. confusum* or *T. castaneum*. The SD 99·9 for adult *S. granarius* (8700 rads) compares with 8600 rads and 7100 rads for the flour beetles. The survival of adult *T. castaneum*, however, (11·2 per cent at 16,000 rads and less than 1 per cent at 19,200 rads) is much higher than for *S. granarius*. The recommended dose, 16,000 rads, would thus leave a small residual population of sterile adults of this species. The amount of damage through feeding by a few surviving adults would, however, be small; Rogers and Hilchey[17] have shown that the rate of feeding of adults of *T. castaneum* at 27,400 rads is more than halved.

CONCLUSIONS

All developmental stages of *T. castaneum* are more resistant to gamma radiation than *T. confusum*. Eggs, larvae, pupae and adults of *T. confusum* require doses of 4400, 5200, 14,500 and 12,800 rads, respectively, for 99·9 per cent kill; the corresponding doses for *T. castaneum* are 10,900, 10,500, 25,800 and 21,500 rads. Production of progeny by *T. confusum* is reduced by 99·9 per cent at 3400, 6400, 4700 and 7100 rads, respectively. Doses of 8600, 8100, 6200 and 8600 rads are required to obtain this reduction in *T. castaneum*. Fertility of adults of both species may be recovered to some extent after irradiation with substerilising doses; there is no redevelopment of fertility in insects receiving doses approaching the evaluated commercial dose of 16,000 rads. This dose controls both species of *Tribolium* although a small residual population of sterile *T. castaneum* adults survive.

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PAPER IX

Susceptibility of the Saw-toothed Grain Beetle, *Oryzaephilus surinamensis* (L.) to Gamma Radiation

D. J. JEFFERIES

Entomological studies into the use of ionising radiations for the control of insects infesting grain have been largely concerned with the susceptibility of the grain weevil, *Sitophilus granarius* (L.), species of lesser importance having received little attention. The saw-toothed grain beetle, *Oryzaephilus surinamensis*, is more resistant to gamma radiation than *S. granarius*; 15,300 rads is required for 99·9 per cent sterility of adults and calculations show that at 30°C, the sterilising dose for weevils (16,000 rads) controls *O. surinamensis* for a period of 8–12 weeks. The efficacy of this dose under conditions likely to be experienced in commercial practice is discussed.

INTRODUCTION

The saw-toothed grain beetle, *O. surinamensis*, is a common pest of stored grain and cereal products in the tropics and is continually introduced by trade into temperate countries. It is usually found as a secondary pest on grain damaged by other insects, e.g. the grain weevil, *S. granarius*, as the larvae cannot develop on sound grains.[1] At present, control is achieved by contact insecticides and fumigants.

In appraising the possible use of ionising radiations for disinfestation of stored cereals[2–5], only limited tests of susceptibility have been reported on the saw-toothed grain beetle. The present study was carried out to examine the susceptibility to gamma radiation of all developmental stages with particular regard to the degree of control afforded by the recommended dose for control of weevils.[6] Subsidiary experiments were also carried out to examine the susceptibility of the sexes and the fertility of the F1 and F2 generations.

EXPERIMENTAL*

Using a laboratory strain of *O. surinamensis*, immature stages for irradiation were obtained by placing samples of 50 eggs on 3 g of lightly cracked Manitoba wheat in small ventilated polythene tubes and incubating at 30 °C and 60 per cent r.h. Eggs, larvae and early and late pupae were treated at 1–2, 10–11, 20–21 and 22–23 days after oviposition, respectively. Adults, 0–14 days old, were irradiated in samples of 50 on 3 g of cracked wheat. Irradiations were carried out in a ^{60}Co source[7] at 30 °C and at 15 dose levels, with 10 samples of immature stages and 5 of adults at each dose. Doses ranged from 1030 to 23,000 rads and were all given at 4000 rads/hr. After irradiation the immature stages were retained in the tubes until pupation when pairs of samples were placed on 100 g of cracked wheat in culture jars. Samples of adults were placed separately on 100 g of cracked wheat immediately after irradiation. Examinations and transfers to fresh grain were made at weekly intervals for a period of 8 weeks, the first examination being made 1 week after irradiation of adults and 2 weeks after the start of emergence for immature stages. The data for emergence and survival (Fig. 1) was corrected for natural mortality[8] and examined by Probit analysis. The yield of progeny in 8 weeks was corrected for variations in parent density to that of 50/100 g wheat (correction curve from AERE-R 3891) and the relationship with dose (Fig. 2) treated as an exponential function.

RESULTS

Emergence of adults

The doses for 50 per cent reduction in emergence (Table 1) show that larvae were slightly more sensitive to radiation than eggs ($P < 0{\cdot}1$ per cent) but the doses for 99·9 per cent reduction were similar. With 19,720 rads, the highest dose used on pupae, emergence from early pupae was reduced by 84 per cent and from late pupae by 30 per cent; it was therefore impossible to obtain a reliable dose for 99·9 per cent reduction for this stage. At the lower doses, 1030 and 2050 rads, emergence from late pupae was 109 per cent and 108 per cent respectively, significantly greater than the control ($P < 5$ per cent; $P < 2$ per cent). More adults than in the control were also obtained with irradiated early pupae, 103 per cent (1030 rads) and 102 per cent (2050 rads), but this increase was too small to be significant. The response at low doses would thus indicate an effect of stimulation.

* Details of experimental technique, together with biological data, are given in U.K.A.E.A. Research Group Report, AERE-R 3891.

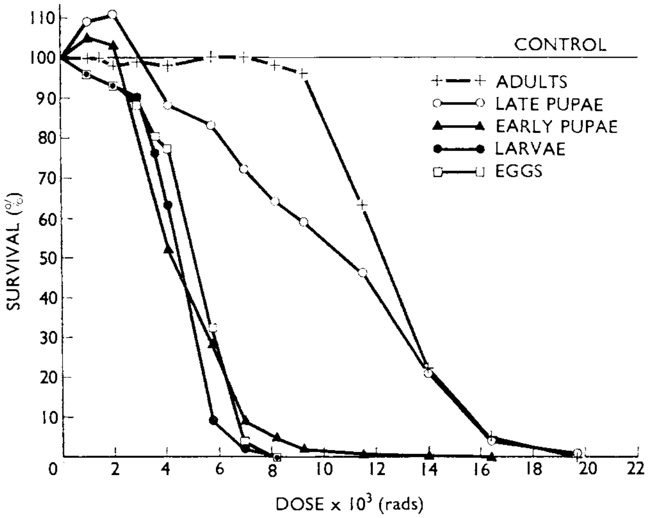


FIG. 1. Relationships between dose and percentage survival at 3 weeks from emergence of immature stages and 2 weeks from irradiation of adults.

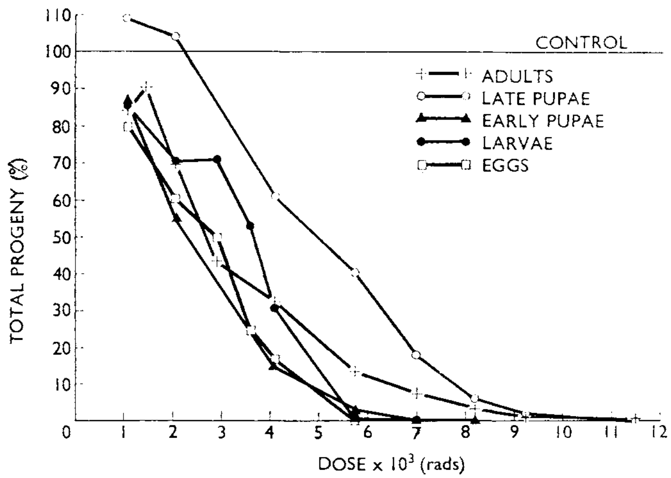


FIG. 2. Relationships between dose and total progeny produced over a period of 8 weeks. (Density standardised to 50 adults/100 g grain.)

TABLE 1

Doses required for 50 per cent and 99·9 per cent reduction in emergence, survival at 3 weeks (from emergence of immature stages and from irradiation of adults) and progeny production of O. surinamensis. *The LD and SD 99·9's for* Sitophilus granarius[9] *are shown for purposes of comparison (16,500 rep ≡ 16,000 rads)*

	Stage	Estimated dose for 50% reduction in emergence/survival (LD 50) Log dose ± S.E.	LD 50 (rads)	Estimated dose for 99·9% reduction in emergence/survival (LD 99·9) Log dose ± S.E.	LD 99·9 (rads)	*Sitophilus*[9] *granarius* LD 99·9 (rep)
Emergence	Eggs	3·737 ± 0·017	5450	4·011 ± 0·049	10,270	6060
	Larvae	3·664 ± 0·006	4610	3·955 ± 0·026	9030	4730
	Early Pupae	3·753 ± 0·046	5660			
Survival	Eggs	3·705 ± 0·012	5070	3·984 ± 0·033	9650	4280
	Larvae	3·642 ± 0·005	4380	3·935 ± 0·023	8620	4350
	Early Pupae	3·634 ± 0·017	4310	4·159 ± 0·038	14,430	
	Late Pupae	4·011 ± 0·018	10,250	4·489 ± 0·064	30,840	8810
	Adults	4·086 ± 0·005	12,190	4·315 ± 0·013	20,650	9200
		Estimated dose for 50% reduction in progeny (SD 50) Dose ± S.E. (rads)		Estimated dose for 99·9% reduction in progeny (SD 99·9) Dose ± S.E. (rads)		*Sitophilus*[9] *granarius* SD 99·9 (rep)
Fertility	Eggs	2300 ± 650		7980 ± 1930		2350
	Larvae	2630 ± 740		6530 ± 1550		4210
	Early Pupae	2210 ± 450		7700 ± 1080		
	Late Pupae	4640 ± 700		12,000 ± 1480		4700
	Adults	2570 ± 230		15,280 ± 580		8680

Survival

Comparison in Table 1 of the doses required for 50 per cent reduction in emergence and survival of immature stages shows that mortality due to irradiation occurred both before and after emergence. Radiation induced mortality was complete by 3 weeks after the start of emergence of immature stages and 2 weeks after irradiation of adults.

The doses for 50 per cent kill (LD 50's, Table 1) show that larvae and early pupae were more sensitive to radiation than eggs ($P < 0{\cdot}1$ per cent). With the 2 days' incubation from early to late pupae, the LD 50 rose markedly from 4300–10,200 rads and further increased to 12,200 rads with the irradiation of adults. The LD 99·9 for late pupae (30,800 rads) was, however, higher than that for adults (20,600 rads), this reversal being caused by differences in the slopes of the regression lines.

Fertility

The five control samples of each stage produced a total of 22,280 ± 1170 progeny in 8 weeks. The doses for 50 per cent reduction in fertility (SD 50's, Table 1) show that eggs, larvae and early pupae were of similar sensitivity; the SD 50 increased sharply from 2200 to 4600 rads with 2 days' incubation from early to late pupae, but fell again to 2600 rads for adults. In contrast with the lethal dose for 99·9 per cent, the SD 99·9 for adults was higher than for late pupae.

Comparison (Table 2) between the yield of progeny obtained from irradiated immature stages during the first and last of the 8 weeks of oviposition shows a decrease in fertility with age – more marked in irradiated insects than in controls. The same applies to irratiated adults treated with doses above 5000 rads. Accordingly there is no evidence of redevelopment of fertility in an irradiated population of *Oryzaephilus*; indeed, at doses above 13,970 rads, fertile eggs were produced for only a few days. The experiments on the susceptibility of the sexes, described below, indicate that the female is responsible for limiting population increase.

Fertility of the F1 and F2 generations

Fertility of the progeny of irradiated adult beetles was examined by allowing them to oviposit for 1 week at a density of 50/1 kg of grain. Two samples were used at each dose. Irradiation of the parent generation with 1, 3, 5, 7, 9 and 16×10^3 rads had a marked effect on the fertility of the F1 generation; the progeny per parent of the F1 generation was, respectively, 108, 50, 51, 49, 48 and 35 per cent of the control. Fertility of the F2 generation was also reduced, being respectively 85, 45, 41, 57, 39 and 35 per cent of the control. Radiation damage was thus passed on to subsequent generations.

TABLE 2

The number of progeny per parent during the first, second (adults only) and last of the 8 weeks of oviposition expressed as a percentage of the control. Dash signifies total death of parents

Dose in rads	Eggs		Larvae		Early pupae		Late pupae		Adults		
	Week of oviposition										
	1st	8th	1st	8th	1st	8th	1st	8th	1st	2nd	8th
1030	85	84	98	91	80	51	90	91	69	90	77
1440									71	101	90
2050	72	70	89	79	61	37	82	76	55	79	70
2880	55	51	91	69					36	51	41
3590	41	24	81	58							
4110	25	18	60	50	29	9	53	47	27	41	27
5750	2	0	5	12	9	0	23	29	16	19	11
6980	0	0	<1	0	6	0	10	13	9	11	5
8210	—	—	—	—	3	0	7	2	7	5	3
9240	—	—	—	—	0	0	4	2	4	3	1
11,500					0	0	1	0	2	<1	0
13,970					0	0	<1	0	2	0	0
16,430					—	—	0	0	1	0	0
19,720					—	—	0	0	<1	0	0
23,000									0	0	0

The effect of mating normal and irradiated stock

The susceptibility of the sexes was examined by irradiating virgin adults and setting up the following combinations of two females and one male. Three samples were used in each treatment. The experiment was also repeated at 4110 rads using mated adults.

Combination	Male Female	Dose
(1)	Irradiated × Irradiated;	2050, 4110 and 6980 rads
(2)	Irradiated × Normal;	2050, 4110 and 6980 rads
(3)	Normal × Irradiated;	2050, 4110 and 6980 rads
(4)	Normal × Normal;	Control

Analysis of variance of the number of progeny per female during the first week's oviposition showed that there was no significant difference between the fertilities of combinations 2 and 3, viz. when either the males or females were treated. In both these combinations, fertility was lower than when neither

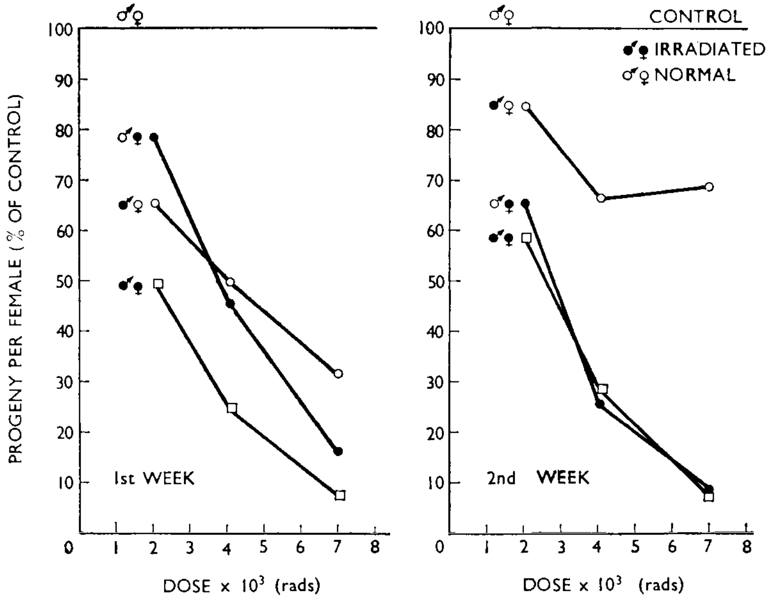


FIG. 3. The effect of mating normal and irradiated stock on the number of progeny produced during the first and second weeks after irradiation.

● Normal Male × Irradiated Female
○ Irradiated Male × Normal Female
□ Irradiated Male × Irradiated Female

sex was treated (combination 4) but significantly higher ($P < 0{\cdot}1$ per cent) than when both sexes were treated (combination 1) (Fig. 3). Thus mature sperm and eggs involved in zygote formation during the first week after irradiation are equally susceptible, and both sexes contribute towards the sterility of the irradiated population. A similar analysis for the second week's oviposition showed no significant difference between the fertilities of combinations 1 and 3, both being significantly lower ($P < 0{\cdot}1$ per cent) than combination 2 (Fig. 3). Thus during the second week after irradiation, males treated with doses of 7000 rads and below regain most of their fertility, whereas the fertility of females remains reduced.

The results for combinations 1, 2 and 3 with insects which had mated before irradiation (with 4110 rads) were, respectively, 10, 44 and 39 per cent (of control) during the first week and 34, 86 and 32 per cent during the second week's oviposition. These results are not significantly different from those obtained with virgin stock. Thus irradiated males are able to reduce the fertility of previously mated, unirradiated females, which suggests that matings are either required very frequently, or spermatozoa of irradiated males competes with or replaces spermatozoa previously inseminated.

DISCUSSION

The experiments described show that gamma irradiation reduces the emergence, survival and fertility of *O.surinamensis* to an extent dependent upon the physiological age and sex of the insect. Eggs, larvae and early pupae are of similar sensitivity, but with development from early to late pupae there is a two-fold increase in resistance which is retained during adult life. In populations treated with sub-sterilising doses, reduced fertility may be attributed entirely to irradiated females, the males having regained most of their fertility within a week of irradiation. Females do not regain fertility. At doses of 3000 rads and above there is a 50 per cent reduction in fertility of the F1 and F2 generations probably due to chromosome translocations passed on from their irradiated progenitors. The number of generations required to regain normal reproductive integrity would depend on the precise nature of the translocation. Discrepancies between the present results and previous work with respect to the LD 50[10] and rate of mortality[11,12,13] of *O.surinamensis* after irradiation are probably caused by different dose rates[14] and differences in post-irradiation culture temperature.[15]

A comparison of the doses required for 99·9 per cent reduction in survival and fertility of *S.granarius* and *O.surinamensis* (Table 1) suggests that the recommended dose for weevils may not be completely effective for the control of *Oryzaephilus.* The regression equations relating survival and fertility with dose enable one to estimate the probable effect of 16,000 rads on a population of this species. It can be shown that in a population with stable age-distribution[16] only 4 per cent of the insects are adults (calculated from an intrinsic rate of increase per week of 0·771[17] and survival data from AERE R-3891). Thus a hypothetical population of 25,000 insects containing 1000 adults was taken for purposes of calculating subsequent changes in population. If this were left untreated, progeny production would be continuous: Howe[17] gives a value of 2·16 for the weekly self-multiplicative rate of increase of *O.surinamensis* at 30 °C. Using this figure, an initial infestation of 25,000 insects with unlimited food would give rise to more than 2500 million in 15 weeks. After irradiation, progeny production is not continuous, so the changes in population can be timed. Since 16,000 rads is completely sterilising to immature stages (Table 2), only adults are capable of producing an F1 generation; calculation shows that the size of the F1 generation produced by irradiated adults in 8 weeks would be 0·093 per cent of the control. In the same period, unirradiated adults, under favourable conditions of low density, each produce 141 adult progeny (a mean of 17·6 progeny/parent/week; biological data from AERE R-3891); 1000 adults would thus produce an F1 generation of 141,000, but only 130 after treatment with 16,000 rads. At high doses all progeny result from oviposition in the first week after irradiation (Table 2). All would have

emerged by 4–5 weeks and allowing 5 days for maturation,[17] eggs are laid at 5–6 weeks. As the progeny (F1) of parents irradiated at 16,000 rads produce only 35 per cent of the offspring produced by controls, then only 800 insects (F2) would be produced by oviposition in the first week. The population would then increase by 800 a week from the sixth to the eleventh weeks after which large population increases would occur with the production of the F3 generation. (For these calculations the number of immature stages present is taken to be similar to the number of adult progeny emerging, as mortality at low densities is almost completely confined to eggs; percentage hatch 89 ± 2 per cent; percentage adult emergence 87 $\pm$ 1 per cent; biological data from AERE-R 3891). Thus Table 3 shows that a dose of 16,000 rads may control an infestation of *O. surinamensis* for a period of only 12 weeks; subsequently the population rises above the initial number of 25,000. Nevertheless, 15 weeks after irradiation the infestation would still be only 0·003 per cent of that produced in the absence of treatment. The period of control as calculated here is much reduced if the population does not have a stable age-distribution. The ratio of immature to adult can be as low as 2 to 1[18] and the number irradiated (a population of 3000 insects containing 1000 adults) would then be reached in 8 weeks.

A number of factors have been omitted from these calculations which, if included, might give greater efficacy to the control achieved with 16,000 rads. Firstly, adult mortality, other than radiation-induced mortality, has not been taken into account. Again, temperatures lower than 30 °C retard development and lower the production of progeny; at 25 °C the time for immature development is increased from 28 to 33 days[17] and the rate of oviposition is decreased from 6–10[17] to 1[19] egg/day. This increases the period of control to 23 weeks for a population with a 2 : 1 immature : adult ratio and for much longer if the ratio is 19 : 1. Dose rate also influences the radiation response such that at intensities likely to be used in practice, considerably above the 4000 rads/hr used in these experiments, a higher mortality and lower level of residual fertility at 16,000 rads is likely to be achieved.[14] Furthermore, only grain damaged by other insects would support the rapid rate of population increase assumed in these calculations; 16,000 rads would considerably reduce damage by weevils and make the entry of *Oryzaephilus* larvae less favourable. While these experiments have been carried out with a laboratory strain of *O. surinamensis*, wild strains remain to be tested; studies on a number of species taken from infestations in storage[20,21] indicate their greater resistance, at least to killing by irradiation. On balance, then, it may be stated that the calculated period of 8–12 weeks is probably the minimum period of control likely to be experienced; in practice, 16,000 rads may be considerably more effective when applied as an industrial irradiation process.

TABLE 3

Estimated population size at intervals after irradiation of 1000 adults with 16,000 rads

Generation	Stage	Weeks after irradiation										
		5	6	7	8	9	10	11	12	13	14	15
P1 (irradiated)	Adult	60	60	60	60	60	60	60	60	60	60	60
F1	Adult	130	130	130	130	130	130	130	130	130	130	130
F2	Immature		800	1600	2400	3200	3200	3200	3200	3200	3200	3200
	adult						800	1600	2400	3200	4000	4800
F3	Immature							5000	15,000	30,000	50,000	70,000
	adult											5000
Total		190	990	1790	2590	3390	4190	9990	20,790	36,590	57,390	83,190

CONCLUSIONS

1. Eggs, larvae, early and late pupae and adults of *O. surinamensis* require doses of 9600, 8600, 14,400, 30,800 and 20,600 rads, respectively, for 99·9 per cent kill. Production of progeny is reduced by 99·9 per cent by 8000, 6500, 7700, 12,000 and 15,300 rads, respectively. Sterility of irradiated populations is almost entirely due to the irradiated females; males regain most of their fertility by the second week after irradiation. Females remain sterile and there is no recovery of normal progeny production. Radiation damage is passed on to later generations and results in reduced fertility.
2. Calculations show that at 30 °C, 16,000 rads controls *O. surinamensis* for 8–12 weeks after which the population rises above the initial number irradiated; at 15 weeks the calculated infestation is 0·003 per cent of the number produced in the absence of irradiation. Several factors indicate that 16,000 rads, applied in commercial practice, is likely to be more effective in controlling *O. surinamensis* than the calculations suggest.

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PAPER X

Susceptibility of Australian Strains of *Sitophilus* and *Tribolium* Species to Gamma Radiation*

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Seventeen wild strains of *Sitophilus* and *Tribolium* species, found naturally infesting grain and cereal products in Australia, are compared with eight laboratory strains in their susceptibility to killing and sterilisation by gamma irradiation from ^{60}Co. The differences obtained are of insufficient magnitude to influence the efficacy of the dose evaluated for radiation disinfestation of grain if applied at port terminals in Australia.

INTRODUCTION

Ionising radiations for the control of insects in grain are being considered[1] as a complement to existing chemical methods of disinfestation. Radiation treatment is suited to countries which handle large quantities of grain in bulk at port terminals equipped with mechanical handling, both importing countries, which need to conserve food supplies for home requirements, and large producing countries, such as Argentina and Australia, where purity of consignments for export is an important consideration.

Using principally gamma radiation, considerable information is already available on the relative susceptibilities of developmental stages of the major grain-infesting species:[2,3,4] Most of the tests have been carried out on laboratory strains, although wild strains of grain weevil, imported from various parts of the world, have also been examined.[5] A dose level for effective control has been evaluated, and the environmental factors which modify susceptibility of the insects to irradiation have also been studied.[6,7,8] The present investigation was carried out to examine the range of suscep-

* This investigation was carried out while the author was at the Wantage Research Laboratory, Isotope Research Division (A.E.R.E.), Wantage, Berks, U.K.

tibility found in strains of three species of weevil and two species of flour beetle naturally infesting stored cereals in Australia, viz. *Sitophilus granarius*, *Sitophilus zeamais*,* *Sitophilus oryzae*,† *Tribolium confusum* and *Tribolium castaneum*.

EXPERIMENTAL

Strains of the five species, usually collections of about 20 adults, were taken from mills, silos and stores in New South Wales and Queensland shortly before the tests were conducted. For comparison, laboratory strains, with the exception of *S. oryzae*, were obtained from the Pest Infestation Laboratory, A.R.C., Slough, kept in culture and used in experiments at the Wantage Research Laboratory for several years. A Canadian strain of *S. granarius*, "Manitoba wild", also obtained from Slough, was included in the series. Laboratory strains of *S. oryzae* and additional laboratory strains of the two species of flour beetle were obtained from Sydney, N.S.W., where they had been maintained in culture for 1–2 years.

The strains of *Sitophilus* were reared in paper sacks, as used by Bull and Cornwell,[7] each containing 1 kg of cleaned Manitoba No. 2 wheat, and inoculated with 1000–2000 adults which were allowed to oviposit for about a week. The first progeny to emerge were discarded together with the parents, and the adults were then removed to age for irradiation; 7–11 days for *S. zeamais* and *S. oryzae*, and 9–14 days for *S. granarius*. The strains of *Tribolium* were reared in jars by inoculating 1 kg of wholemeal flour, milled from Manitoba wheat, with about 3000 eggs. The adults obtained were 4–11 days old at irradiation. The laboratory strain of *T. confusum* from the U.K. was also tested when 21–28 days old.

All species were treated in batches of 100 adults at 26 °C in ventilated tubes in a ^{60}Co source[9] at 4000 rads/hr, those of *Sitophilus* on 3 g of wheat and the species of *Tribolium* on 3 g of flour. Each strain was tested at 15 dose levels (750–25,000 rads), and two samples retained as controls. To eliminate any variation in treatment all strains of one species were irradiated on the same day; after irradiation, the weevils were placed with 1 kg of wheat and the flour beetles with 100 g of wholemeal. They were examined periodically for mortality and transferred to fresh food which was then incubated for progeny; those of *S. zeamais* and *S. oryzae* were counted at 8 weeks, and those of *S. granarius* at 9 weeks. The progeny of *Tribolium* were recorded 6–7 weeks after transfer.

* Previously known as *S. oryzae* L. (large strain).

† Previously known as *S. sasakii* Takahasi.

RESULTS

Onset of mortality

The interval between irradiation and onset of death varied appreciably between strains and between the five species (Table 1). Death of the Australian strains of *S. granarius* and *S. zeamais* was delayed by about 2 days compared with the laboratory strains, and the delayed response period for the "Manitoba wild" was increased by 4 days. No difference in response could be discerned between laboratory and wild strains of *S. oryzae*; there was also little

TABLE 1

Delayed response to irradiation (days for onset of death) for laboratory and wild strains

Species	No. of strains tested	Origin Laboratory (days)	Origin Wild Australian (days)
S. granarius	6	8	10 (12*)
S. zeamais	3	6–8	8–10
S. oryzae	7	8–10	
T. confusum	4	12 (9†)	12 (12–15)
T. castaneum	5	15	12 (12–15)

* "Manitoba wild" strain
† 3–4 weeks old at irradiation
( ) exceptions to the majority

difference between strains of *T. confusum*, but the laboratory strain of this species, irradiated when 3–4 weeks old, began to die 3 days earlier than those irradiated at a week old. *T. castaneum* contrasted sharply with the other species since the delayed response period for wild strains was in most cases *reduced* by about 3 days compared with the laboratory strains. Individuals of *S. granarius*, *S. zeamais* and *T. castaneum*, which remained alive 21 days after irradiation, suffered no further mortality; those of *S. oryzae* and *T. confusum* which survived 16 and 24 days, respectively, also remained alive during further observation. The susceptibility of strains was thus compared at these intervals after irradiation.

Mortality

Probit/log dose transformations were used to compare the mortalities of strains after correcting for natural mortality. Tests of significance were made only at the LD 50's since in many cases the slopes of the regression lines differed between strains of the same species.

S. granarius

The dose for 50 per cent kill of the laboratory strain (5000 rads) was significantly lower ($P < 0{\cdot}1$ per cent) than that for the four Australian strains (6800–7500 rads); some of these also differed significantly from one another (Fig. 1). The slope of the regression for the "Manitoba wild" strain was markedly steeper ($P < 1$ per cent) than for other strains of *S. granarius*, with the result that its LD 99·9 (7800 rads) was far below that of the others (about 14,000 rads), (Table 2).

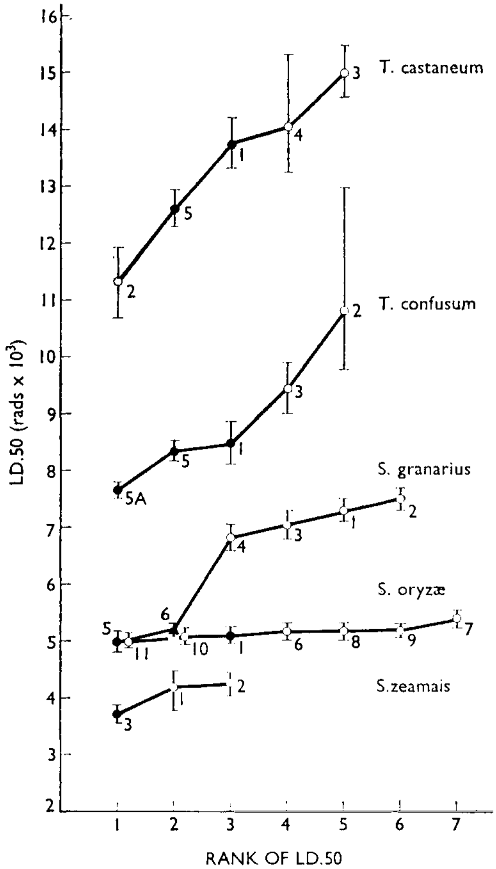


FIG. 1. Strains (numbered) of the five species of *Sitophilus* and *Tribolium* placed in order of susceptibility to killing by gamma radiation. LD 50's and 95 per cent confidence limits of *S. granarius*, *S. zeamais* and *T. castaneum* calculated for 21 days after irradiation; *S. oryzae* at 16 days and *T. confusum* at 24 days.

Laboratory strains ●
"Manitoba wild" strain of *S. granarius* ▲
Australian wild strains ○
5A 3–4 week old samples of *T. confusum*

S. zeamais

The LD 50's of the two wild strains were similar (4200 and 4300 rads) and higher ($P < 1$ per cent) than that of the laboratory strain (3700 rads). The LD 99·9's were also considerably greater (Table 2).

S. oryzae

S. oryzae provided LD 50's all of similar magnitude, the range extending only 400 rads for all strains. There was also a close similarity between values for the LD 99·9, (range 800 rads; Table 2).

Tribolium species

In both species of flour beetle the range in susceptibility of wild strains was more pronounced than among the species of *Sitophilus*. A 50 per cent kill of *T. confusum* was obtained with 8500 rads for the laboratory strain from Australia, and with 8350 rads for that from the U.K. When the latter was irradiated at 3–4 weeks old, however, the LD 50 was reduced to 7650 rads. The two wild Australian strains, with LD 50's of 9500 and 10,800 rads, were significantly more resistant ($P < 5$ per cent) than strains from laboratory culture. The LD 99·9's for laboratory strains of *T. confusum* were both near 12,350 rads, lower than 17,600 and 22,200 rads obtained for the two wild strains. The 3–4-week old samples of the laboratory strain had the lowest LD 99·9, namely, 10,500 rads, commensurate with the low value obtained for the LD 50.

The results for *T. castaneum* were similar to the above, with the exception that one of the wild strains (LD 50; 11,300 rads) was considerably more susceptible than both strains from laboratory culture (Australian, 13,700; U.K., 12,600 rads). The remaining two wild strains were, however, significantly more resistant (LD 50's; 14,000 and 15,000 rads). For a 99·9 per cent kill, estimates had to be extrapolated outside the dose range investigated and should therefore be considered with some reserve; they ranged from 21,200 for the laboratory strain from the U.K. to 34,000 for the most resistant wild strain.

Fertility

The effect of irradiation in reducing the numbers of progeny in the F_1 generation was analysed by treating the fertility/dose relationship as an exponential function (log per cent progeny against dose), which provided linear regression lines. Significant differences were again found in the slope of response of the various strains. The SD 50 (Fig. 2) and SD 99·9 (Table 3) were calculated for each strain, but because of considerable variation in the data statistical comparisons were not attempted.

S. granarius

The two strains most resistant to killing by irradiation were, surprisingly, the most susceptible (SD 50; 970 rads) and the most resistant (SD 50;

TABLE 2

LD 99·9 (rads) with 95 per cent confidence limits for laboratory and wild strains of Sitophilus *and* Tribolium *species. Wild strains arranged in descending order of increased resistance at LD 50 (see Fig. 1). The 95 per cent confidence limits for* Tribolium *spp. are omitted, as the LD 99·9 is close to, or extrapolated outside, the dose range*

Strains		Species				
		S. granarius	*S. zeamais*	*S. oryzae*	*T. confusum*	*T. castaneum*
Laboratory	U.K.	13,000 {15,600 / 10,800}	6600 {6900 / 5500}	—	12,300 †(10,500)	21,200
	Aust.	*(7800) {8500 / 7200}	— —	7900 {8400 / 7500}	12,400	29,300
Wild Aust.		13,600 {16,400 / 11,300}	9500 {12,000 / 7500}	8300 {9000 / 7600}	17,600	22,100
		14,500 {17,800 / 11,800}	13,700 {19,100 / 9900}	8000 {9100 / 7100}	22,200	34,500
		13,700 {16,300 / 11,500}		7400 {8100 / 6800}		28,800
		14,500 {16,600 / 12,600}		7900 {8700 / 7200}		
				7500 {8200 / 6900}		
				8200 {9200 / 7300}		

* "Manitoba wild" strain
† U.K. strain 3–4 weeks old at irradiation

1750 rads) to sterilisation. This range embraced the SD 50's (1370 and 1050 rads) for the laboratory and "Manitoba wild" strains, respectively. Strains most susceptible to killing by irradiation were not, therefore, the most

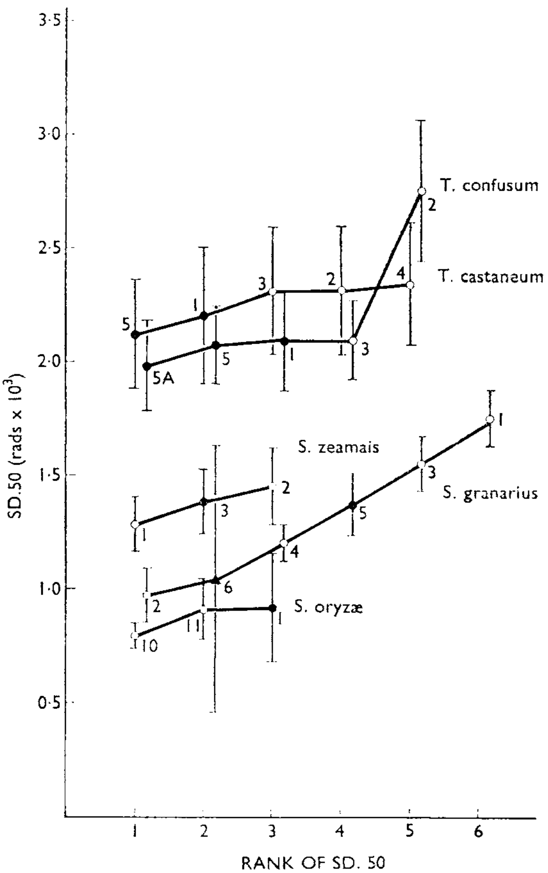


FIG. 2. Strains (numbered) of the five species of *Sitophilus* and *Tribolium* placed in order of susceptibility to sterilisation by gamma radiation. SD 50's and standard errors calculated for all species on progeny, as percentage of control, produced in the four weeks after irradiation.

Laboratory strains ●
"Manitoba wild" strain of *S. granarius* ▲
Australian wild strains ○
5A 3–4 weeks old samples of *T. confusum*

susceptible to sterilisation. The SD 99·9's (Table 3) ranged from 9000 rads for the U.K. laboratory strain to 11,000 rads for the most resistant wild strain.

S. zeamais

Little difference in fertility was found between the three strains at the SD 50 and SD 99·9; the laboratory strain was of intermediate susceptibility at the SD 50.

TABLE 3

SD 99·9 (rads) of laboratory and wild strains of Sitophilus *and* Tribolium *species. Wild strains arranged in descending order of increased resistance at the SD 50 (see Fig. 2)*

Strains		Species				
		S. granarius	*S. zeamais*	*S. oryzae*	*T. confusum*	*T. castaneum*
Laboratory	U.K.	9000	9000	—	7700 †(8100)	9900
	Aust.	*(9800)	—	7500	7600	9500
Wild Aust.		10,400 10,200 10,700 11,000	7500 8200	7400 7200	8400 7900	9700 9200 9700

* "Manitoba wild" strain
† U.K. strain, 3–4 weeks old at irradiation

S. oryzae

Only three strains of *S. oryzae* were tested satisfactorily for fertility; SD 50's ranged from 800 to 920 rads, with the laboratory strain appearing slightly more resistant. *S. oryzae* is undoubtedly the most susceptible species of *Sitophilus* to radiation sterilisation at both the 50 per cent sterilising dose, and at the SD 99·9.

Tribolium species

The narrow range among strains of the two species of flour beetle in susceptibility to sterilisation contrasts markedly with their wide range in tolerance to killing by irradiation. In both species, the laboratory strains were the most susceptible at the SD 50; values for *T. confusum* increased from about 2000 rads for the U.K. laboratory strain (whether tested as 1-week old or 3–4 week old adults) to 2750 rads for the wild strain with the highest LD 50. The SD 99·9's ranged from near 7700 rads for both laboratory strains to 8400 for a strain from the field (Table 3).

The laboratory strains of *T. castaneum* (Fig. 2) were similar in susceptibility to the wild strains; the SD 99·9's were all extremely close (9200–9800 rads), with the laboratory strain from the U.K. apparently the most resistant.

DISCUSSION

This investigation clearly substantiates the existence of differences in radiation susceptibility between laboratory and wild strains of the grain weevil,[5] in addition to establishing differences between strains of other species. "Onset of death", is a parameter of susceptibility which is not easily measured; in four species there was about a 2-day difference of "impending mortality" between laboratory and wild strains, which can be attributed to the greater resistance of the latter. The results for *T. castaneum* are, however, anomalous and the parameter used in previous work[5] viz. time for 50 per cent kill, is likely to provide a more critical measurement.

Little difference could be found between strains of *S. oryzae* and *S. zeamais* to the lethal effects of irradiation. Wild strains of *S. granarius* and of the two species of *Tribolium* were generally more resistant than the laboratory strains; this difference appears greater for *Tribolium* species than for *S. granarius*, but the percentage increase in LD 50 of "wild" over "laboratory" is, in fact, smaller for *Tribolium* than for *S. granarius*. Thus, among all the species examined, *S. granarius* shows the greatest variability. This is possibly because the particular laboratory strains of *S. granarius* used in this study have been cultured for a large number of years. Similar results have also been found with insecticides.[10] The LD 50's for *S. oryzae* and *S. zeamais* were remarkably uniform, an indication perhaps of low genetic variability, or a relatively short period of subjection to laboratory culture. Poor culture conditions can increase radiation susceptibility considerably,[7] but the conditions of culture used in these experiments were rigorously standardised. Until recently the species referred to in this paper as *S. oryzae* was known as *S. sasakii*; the results obtained from this work show that adults of *S. oryzae* are not only more resistant to irradiation than *S. zeamais* (previously known as large strain *S. oryzae*), but the slopes of the log/dose probit relationships are also different – an indication of a physiological separation of the two species.

Differences between strains in response to sterilisation by irradiation were generally small, the greatest differences being found in *S. granarius*. Consistent with previous work, using only laboratory strains,[1,4] the *Tribolium* species were the most resistant at the SD 50. Effective control of insects by irradiation, however, depends upon a dose which provides complete sterilisation; the SD 99·9 for wild strains of *S. granarius* was higher than for the other four species. As it has been previously demonstrated that both laboratory and wild strains of *S. granarius* can be effectively controlled by 16,000 rads,[8] it follows that the other four species will succumb to similar treatment. Accordingly, there are not likely to be any serious problems in the application of radiation disinfestation with the evaluated dose against strains of *Sitophilus* and *Tribolium* encountered in Australia.

CONCLUSIONS

1. No differences could be detected between laboratory and wild strains of *S. oryzae* and *S. zeamais* in the lethal effect of gamma radiation. Wild strains of *S. granarius*, *T. confusum* and *T. castaneum* were more resistant than laboratory strains.
2. In the five species, only slight differences between laboratory and wild strains were found in response to sterilisation by irradiation. Sterilisation at the 99·9 per cent level was obtained with all species and strains at about 10,000 rads.
3. The dose of 16,000 rads, previously evaluated for effective control of *S. granarius*, can therefore be recommended for use in commercial radiation disinfestation of grain in Australia.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the generosity of the U.K. Atomic Energy Authority in making its facilities at the Wantage Research Laboratory available for this work.

For the collection of the Australian strains the author wishes to thank Mr. S. W. Bailey of the C.S.I.R.O., Canberra, Mr. G. Dolezal of the University of New South Wales and members of the Australian Wheat Board and Queensland State Wheat Board.

Thanks are also due to Mr. T. Hobson, Theoretical Physics Division, A.E.R.E., Harwell, Berks., for assistance with the statistical analyses.

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PAPER XI

Some Effects of Gamma Radiation on *Rhizopertha dominica* (F.), *Cadra cautella* (Wlk.), *Plodia interpunctella* (Hübn.) and *Lasioderma serricorne* (F.)

J. B. PENDLEBURY, D. J. JEFFERIES,
E. J. BANHAM and J. O. BULL

Various effects of gamma radiation on four stored-products pests, which infest grain and cereal products in varying degrees, are examined with particular emphasis on susceptibility to radiation sterilisation. *Rhizopertha dominica*, described as the most destructive pest of grain, and *Lasioderma serricorne*, occasionally found infesting cereal products, are effectively sterilised by the dose (16,000 rads) evaluated for the control of large populations of grain weevils. *Cadra cautella* and *Plodia interpunctella*, principally pests of dried fruits, but occasionally imported on infested cereals, are more resistant to radiation sterilisation; 16,000 rads is unlikely to be completely effective for their control.

INTRODUCTION

Detailed studies so far carried out at this laboratory on the susceptibility of stored products pests to gamma radiation have been confined almost entirely to those species most frequently occurring in infested grain: the grain weevil, *Sitophilus granarius*,[1–7] the rice weevil, *Sitophilus zeamais*,[2] the flour beetles, *Tribolium castaneum* and *Tribolium confusum*,[8] and the saw-toothed grain beetle, *Oryzaephilus surinamensis*.[9] These studies have shown that irradiation of large populations of grain weevils with 16,000 rads results in complete sterilisation, and that the same dose can also be recommended for control of *S. zeamais*, *T. confusum* and *T. castaneum*. *O. surinamensis*, the most resistant of the species studied, is incompletely sterilised by 16,000 rads; *Anagasta kühniella*, a pest of flour mills, is also inadequately controlled by this dose.[10]

To complete this series of studies on the radiation susceptibility of grain-infesting species, the tests reported here were carried out on the Lesser Grain Borer *(R. dominica)*, an important pest of grain, particularly of the

dry tropics, the Tropical Warehouse Moth *(Cadra [Ephestia] cautella)*, and two species less frequently encountered on cereals, the Indian Meal Moth *(P. interpunctella)* and the Cigarette Beetle *(L. serricorne)*. These tests were carried out on developmental stages most resistant to ionising radiations, namely as pupae or adults, to evaluate the degree of control obtained by the recommended dose.

METHODS AND MATERIALS

Laboratory strains of the four species were obtained from the Pest Infestation Laboratory, A.R.C., Slough. For the purposes of irradiation, samples of pupae and adults of *R. dominica* were obtained by placing 1000 insects to oviposit on 1 kg of Manitoba No. 2 wheat for 3 days and incubating at 30 °C and 60 per cent r.h. *C. cautella* was irradiated as mixed stages (11 per cent larvae, 64 per cent pupae and 25 per cent adults), obtained from samples of 100 newly-laid eggs sprinkled on to the surface of 50 g of coarse wholemeal flour in 4-in. cube polystyrene boxes, and incubated at 26 °C and 76 per cent r.h. Five per cent by weight of glycerine was added to the culture medium, to accelerate the rate of development.(11) Samples of *P. interpunctella* and *L. serricorne*, containing mostly pupae, were obtained by mixing 250 eggs and 500 eggs, respectively, into 50 g of medium wholemeal in boxes, and incubating at 25 °C and 75 per cent r.h. They were irradiated when about 10 per cent of eggs had matured to adults.

Irradiation

All insects were treated with gamma radiation from ^{60}Co. Since these studies were carried out by independent workers over a period of some months, the conditions at irradiation varied for the different species (Table 1).

Retention after irradiation

The conditions of retention and the observations carried out on the insects after irradiation also varied with the species. Each sample of 50 irradiated adults of *Rhizopertha* was held separately on 100 g of Manitoba wheat. Grains containing pupae of *Rhizopertha* were added to fresh wheat, but pairs of samples were combined after irradiation to provide approximately 50 adults per sample at emergence. Both stages were incubated at 30 °C and 60 per cent r.h. The irradiated adults and those which emerged from the irradiated pupae were transferred to fresh grain weekly for a period of 2 to 9 weeks. Observations on mortality were made on each occasion. The number of progeny produced during the first 3 weeks after irradiation was also recorded.

After irradiation, the samples of flour infested with *C. cautella* and *P. interpunctella* were placed, together with any emerged adults, on the surface of fresh culture media in cardboard trays. Two kg of wholemeal/glycerine mix-

TABLE 1

Irradiation treatments

Conditions	Species			
	R. dominica	*C. cautella*	*P. interpunctella*	*L. serricorne*
Culture medium	approx. 10 g wheat in polytainers (2·5″ × 0·5″)	50 g of wholemeal in 4″ cube polystyrene boxes		
Size of samples	*pupae:* approx. 50 in each of 10 samples/dose *adults:* 50 in each of 5 samples/dose	Samples resulting from incubation of:		
		100 eggs	250 eggs	500 eggs
		8 samples/ dose	2 samples/dose	
Age	*pupae:* 32–35 days from oviposition *adults:* 4–7 days old	mostly as pupae		
Temperature (°C)	30	20		
Dose rate (rads/hr)	4000		13,600	
Dose range (rads)	12 dose levels (1000–20,000)	10 dose levels (3000–20,000)	4 dose levels (10,000–36,000)	

ture was used for *C. cautella* and 1 kg of wholemeal for *P. interpunctella*. The two species were held at 26 °C and 76 per cent r.h., and 25 °C and 70 per cent r.h. respectively. The adult life-span of *Cadra* and *Plodia* under these conditions is short: after 5 and 3 weeks respectively, all adults of the two species had emerged and died. They were counted and sexed; females were dissected to determine whether they had mated and the number of spermatophores in the bursa copulatrix were recorded. The wholemeal on which the adults had laid eggs was incubated for a further 6 weeks to provide an assessment of fertility.

After the treatment of *L. serricorne*, the irradiated flour and insects were removed from each polystyrene box and incubated at 25 °C and 70 per cent r.h. on 0·5 kg of wholemeal in a large culture jar. Adults were removed and counted after 4 weeks, and counts of progeny were made 5 weeks later.

RESULTS

Various observations were made of the effects of irradiation on the four species; emergence, wing deformity, survival, mating behaviour and fertility. Results are detailed for each species separately.

Rhizopertha dominica

Emergence

The numbers of adults which emerged from the irradiated pupae were not reduced by doses up to 20,000 rads.

Survival

The threshold dose for mortality of pupae was about 3000 rads and for adults about 4000 rads (Fig. 1). The mortality response of both stages to gamma radiation was much extended compared with other species;[12] adults obtained from irradiated pupae continued to die up to 7 weeks after irradiation and insects treated in the adult stage continued to die up to 9 weeks. Treatment of both stages with 20,000 rads resulted in a few survivors (1–2 per cent) at 9 weeks. The calculated doses for 50 per cent and 99·9 per cent kill 9 weeks after irradiation were not significantly different for the two stages (pupae 7440 and 24,880 rads; adults 6960 and 25,650 rads).

TABLE 2

Progeny production of irradiated pupae and adults of R. dominica *(as percentage of mean control during the first 3 weeks)*

Dose (rads)	Pupae	Adults
1000	78·2	84·2
1400	66·6	76·4
2800	35·8	36·2
4000	14·7	15·3
5600	5·9	6·3
6800	3·8	2·5
8000	1·3	1·0
9500	0·2	0·3
11,200	0·3	0·1
13,600	0	0
16,000	0	0
20,000	0	0

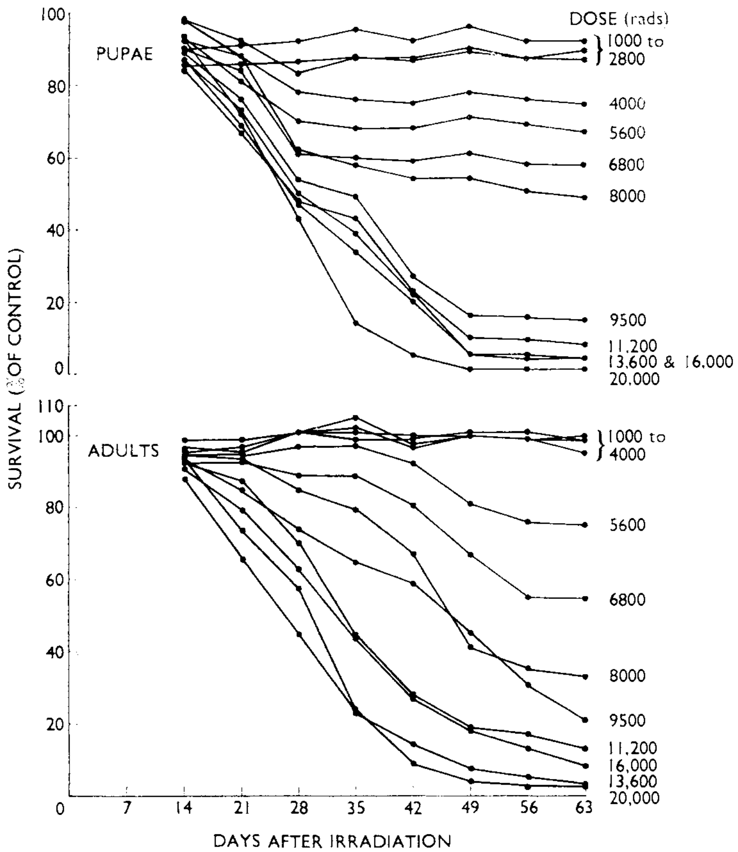


FIG. 1. The effects of 1000 to 20,000 rads on the survival of pupae and adults of *R.dominica* with time after irradiation.

Fertility

In contrast with the long period for radiation damage to culminate in death, there was no delay in the effects of irradiation on fertility; during the first 3 weeks after irradiation the number of viable eggs laid was markedly reduced. Some progeny, however, were produced at doses up to 11,200 rads (Table 2). Again there was no significant difference between pupae and adults in the doses for 50 per cent and 99·9 per cent reduction in progeny (pupae 2240 and 10,750 rads; adults 2140 and 11,240 rads).

Cadra (Ephestia) cautella

No previous radiation studies have been reported on *C.cautella*; the present tests were concerned principally with the susceptibility of the sexes and the effects of irradiation on mating activity.

Emergence

About 25 per cent of adults had emerged in the samples at irradiation, and of the 100 eggs used in each sample, 56 per cent matured to adults in the

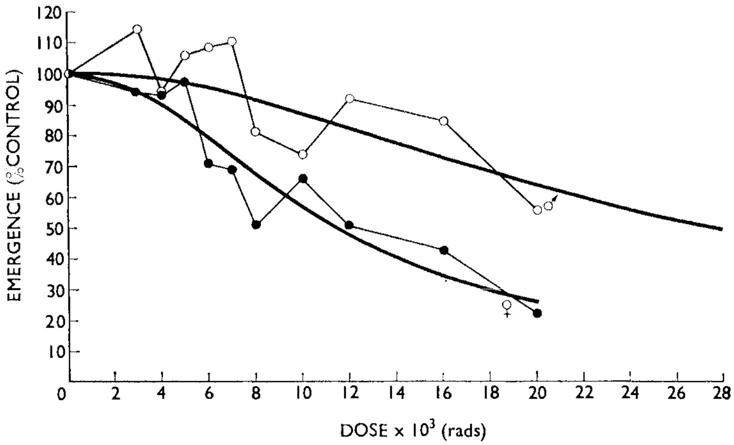


FIG. 2. The effect of dose on emergence of male and female *C. cautella*. Observed data (thin lines), calculated relationships (thick lines).

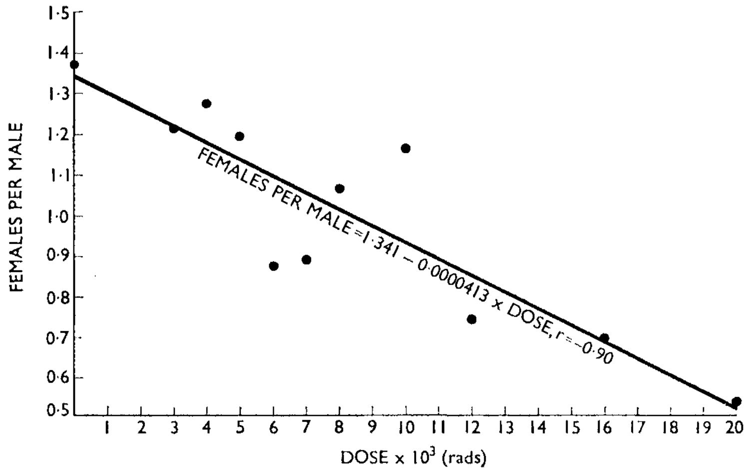


FIG. 3. Relationship between dose and sex ratio of adult *C. cautella*.

controls. A small number of untreated samples were examined 3 and 6 days after the beginning of emergence; 162 males and 15 females emerged during the first 3 days but 122 males and 117 females emerged between 3 and

6 days. A difference in speed of development of the sexes was clearly demonstrated.

Probit analysis of the numbers of males and females which emerged at each dose showed a significant difference in susceptibility between the sexes (Fig. 2); 11,600 rads was required to reduce the numbers of emerging females to 50 per cent, compared with 28,400 rads for males. This difference in susceptibility of the sexes modifies the sex ratio of the emerging adult population with increasing dose ($r = 0{\cdot}90$, $P < 0{\cdot}1$ per cent). The sex ratio of the controls (1 male: 1·37 females) was reduced to unity at 8000 rads and above this dose level there was an excess of males (Fig. 3).

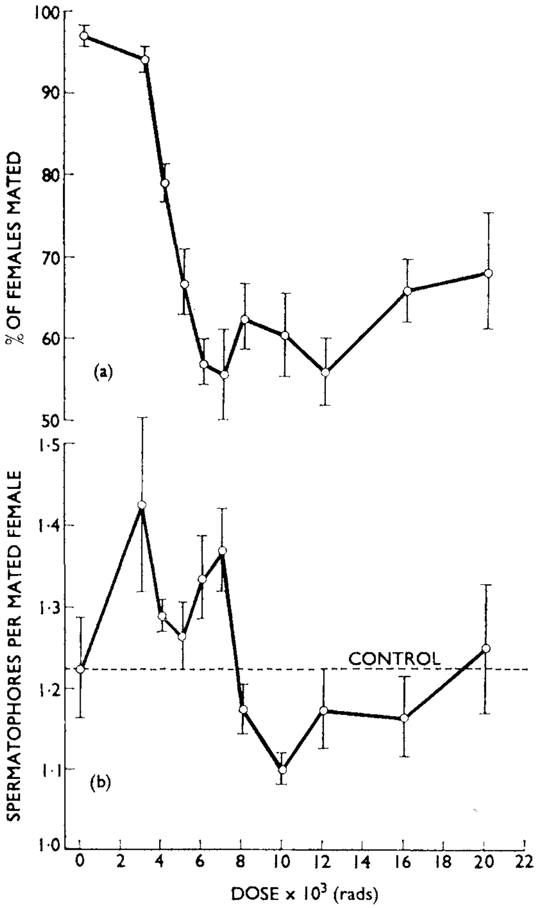


FIG. 4. The effect of dose on the percentage (a) of mated female *C. cautella* and (b) the number of spermatophores per mated female. Vertical lines denote Standard Errors.

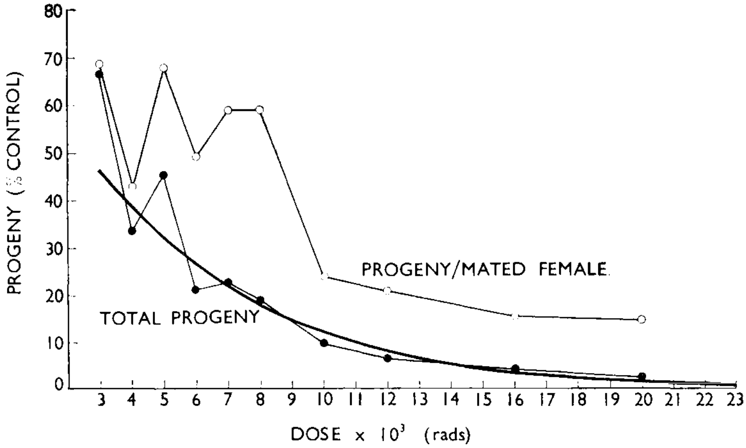


FIG. 5. Reduction in total progeny and progeny per mated female of *C. cautella* with increasing dose. Calculated relationship for total progeny shown by thick line.

Mating activity

Irradiation reduced the number of mated females (Fig. 4a) from 97 per cent in the control to 55 per cent at 7000 rads ($P < 0{\cdot}1$ per cent). At higher doses, the number increased significantly to almost 70 per cent at 20,000 rads ($P < 0{\cdot}1$ per cent). At 3000 rads, the greater number of spermatophores (1·43) per mated female (Fig. 4b) above that of the control (1·23) was not statistically significant. At 10,000 rads, the number of spermatophores (1·11) was significantly reduced ($P < 1$ per cent). Above 10,000 rads there was a gradual increase in the mean number of spermatophores, but the value obtained at 20,000 rads (1·25) was not significantly different from the control. Thus irradiation of *C. cautella* results in a marked loss of mating activity with doses up to 7000–10,000 rads and a slightly increased mating activity between 10,000 and 20,000 rads.

TABLE 3

The effect of gamma radiation on the percentage emergence of male and female P. interpunctella

Dose (rads)	Emergence (% of control)			Sex ratio
	Males	Females	Total	
0	100·0	100·0	100·0	1:0·80
10,000	79·8	75·0	77·6	1:0·76
16,000	70·7	82·5	76·0	1:0·92
20,000	62·6	58·7	60·9	1:0·78
36,000	63·6	70·0	66·5	1:0·88

Fertility

The control samples produced a mean of 1298 progeny, an average of 44·1 progeny per mated female. A 50 per cent reduction in the number of progeny was obtained with 2700 rads and a 99 per cent reduction with 23,500 rads. These doses produced approximately a 25 per cent and 85 per cent reduction in the number of progeny per mated female (Fig. 5).

Plodia interpunctella

Emergence

Failure to incorporate a small amount of glycerine in the rearing medium for *Plodia* resulted in high mortality during development; only 36 per cent of the 250 eggs used to provide samples for irradiation emerged as adults in the control. Treatment with 10,000 and 16,000 rads reduced adult emergence to about 75 per cent of the control, and with doses of 20,000 and 36,000 rads, emergence was reduced to 60–66 per cent. Tests of significance showed no difference in the radiation susceptibility of males and females; the sex ratio of the emerging adults (1 male:0·8 female) was similar for both the control and irradiated samples (Table 3).

TABLE 4

The effect of gamma radiation on wing deformity of adult P. interpunctella

Dose (rads)	Wing deformity (%)			
	Categories			
	1	2	3	4
	→ Increasing deformity →			
0	65	32	3	0
10,000	45	48	6	1
16,000	31	62	7	0
20,000	20	74	4	2
36,000	29	65	5	1

Wing deformity

For the purposes of assessing the possible effects of irradiation on mobility, the emerged adults were classified by the condition of the wings. Four arbitrary groups were defined similar to those used by Bull and Wond:[10]

(1) Wings perfect,
(2) Wings slightly twisted,
(3) Wings very twisted, but fully expanded,
(4) Wings very twisted, and not fully expanded.

The percentage of moths emerging with slightly deformed wings increased with dose (Table 4); no difference was found between the sexes.

Mating activity

Dissection of females for spermatophores (Table 5) showed that doses up to 36,000 rads had no effect on the number of females which mated. The number of spermatophores per mated female increased from 1·74 in the control to 2·83 at the highest dose ($P < 1$ per cent).

TABLE 5

The effects of gamma radiation on the mating activity and fertility of P. interpunctella

Dose (rads)	Mating activity		Fertility (% of control)	
	% Females mated	Spermatophores per mated female	Total progeny	Progeny per mated female
0	100·0	1·74	100·00	100·00
10,000	100·0	2·49	9·90	13·17
16,000	98·4	2·54	0·34	0·40
20,000	100·0	2·75	0·03	0·05
36,000	95·2	2·83	0·59	0·79

Fertility

Unirradiated samples of *Plodia* produced an average of 1611 progeny, a mean of 40·3 progeny per mated female. The data (Table 5) was inadequate to provide a statistical relationship between progeny production and dose; at 10,000 rads progeny were reduced to 10 per cent of the control, and at 16,000 rads and above to about 0·5 per cent.

Lasioderma serricorne

Emergence

About 60 per cent of the 500 eggs developed to adult in the controls. The number of adults which emerged from the irradiated pupae was not reduced by doses up to and including 36,000 rads. The sex ratio of the emerging adults was also unaffected (Table 6).

Fertility

The control cultures produced 9786 progeny per sample, a mean of 70·7 progeny per female. Treatment with doses from 10,000 to 36,000 rads reduced the production of progeny to about 0·01 per cent of the control (Table 7).

TABLE 6

The effect of gamma radiation on the percentage emergence of male and female L. serricorne

Dose (rads)	Emergence (% of control)			Sex ratio
	Males	Females	Total	
0	100·0	100·0	100·0	1:0·90
10,000	112·1	81·1	97·8	1:0·63
16,000	102·9	94·2	98·9	1:0·80
20,000	115·3	93·8	105·5	1:0·70
36,000	115·0	104·3	110·0	1:0·77

TABLE 7

The effect of gamma radiation on the fertility of L. serricorne

Dose (rads)	Fertility (% of control)	
	Total progeny	Progeny per female
0	100·00	100·00
10,000	0·02	0·02
16,000	0·03	0·03
20,000	0	0
36,000	0·01	0·01

DISCUSSION

Emergence from the cultures of immature stages of *R. dominica*, irradiated by Cornwell *et al.*,[12] was markedly reduced. Lack of an effect of 20,000 rads in the present work is adequately explained by the difference in stage of development at the time of irradiation – sensitive immature stages in the earlier work, compared with the more resistant, mature pupae and adults of the present studies. Emergence of *L. serricorne* was unaffected by doses up to 36,000 rads, whereas much lower doses reduced the emergence of both species of moth. Irradiation of *C. cautella* suppressed the emergence of females to a greater extent than males. This can be attributed to the different rates of development, males being at a more advanced physiological stage at irradiation than females.

Wing deformities in *Plodia* occurred in both males and females, and increased in frequency with dose, there being little increase in the proportion of badly deformed adults. Despite these imperfections, there was no loss in

mating activity, as shown by the percentage of females which mated; similar slight imperfections were found[10] not to affect the mating behaviour of *A. kühniella*. In female *P. interpunctella*, the number of inseminated spermatophores increased with dose. In *C. cautella*, the percentage of mated females and the number of spermatophores decreased with treatment up to 10,000 rads, and increased again from 10,000–20,000 rads. The earlier workers with *A. kühniella*,[10] and Bull and Cornwell with *Sitophilus granarius*,[6] have shown that sublethal doses which almost completely sterilise may increase longevity. The greater number of inseminated spermatophores in both moths may thus result from additional matings during an increased life-span. The increase in spermatophores, in female *C. cautella*, could also result from the change in sex ratio. With female *A. kühniella*,[10] maintained with males in different sex ratios, there was, however, only a small difference in the number of inseminated spermatophores.

Only in *R. dominica* was survival examined in detail. Rate of death was very much prolonged, 20,000 rads ultimately producing 98 per cent mortality after 9 weeks. No cytological evidence is available to explain the slow death rate of *Rhizopertha*. The rate of response in the present work was even slower than that observed by Cornwell *et al.*;[12] Fig. 1 shows that adults irradiated with 6800 rads began to die at about 35 days, whereas almost 60 per cent of those treated with 6000 rads in the earlier work had already died. This can probably be explained by differences in the age of adults at irradiation; 4–7 days old in the present work, but certainly older than this in the earlier study. That *R. dominica* is more resistant to killing by irradiation than most other pests of stored products, supports the findings of Proctor *et al.*[13]

Examination of the sterilising response to irradiation shows that a dose of 16,000 rads reduced progeny production by 100 per cent in *R. dominica*, 96 per cent in *C. cautella*, 99·7 per cent in *P. interpunctella* and 99·9 per cent in *L. serricorne* (Table 8). In *C. cautella* the principal factors responsible for sterility appear to be a reduction in (1) emergence of both sexes of adult,

TABLE 8

The effects of 16,000 rads on the emergence and progeny production of R. dominica, C. cautella, P. interpunctella *and* L. serricorne

Species	Emergence (% of control)			Progeny (% of control)
	Males	Females	Total	
*R. dominica**	—	—	100·0	0·05
*C. cautella**	73·2	34·8	50·5	4·12
P. interpunctella	70·7	82·5	76·0	0·34
L. serricorne	102·9	94·2	98·9	0·03

* calculated values

(2) the proportion of successful copulations, and (3) the fertility of those females which mate successfully. In *P. interpunctella*, mating activity was unaffected by irradiation, and the reduction in progeny must be attributed solely to a reduction in emergence and fertility.* In *L. serricorne*, the number of emerged females was unaffected by irradiation; the mean number of progeny per female and total progeny were very similar, suggesting that mating activity was also unaffected. The reduction in progeny must therefore be caused solely by a reduction in viable gametes.

Finally, Lepidoptera appear to be more resistant to the lethal and sterilising effects of gamma radiation than representatives of other orders. Similar observations have been made by Nicholas and Wiant(14) and by workers at this laboratory with *A. kühniella*. Large doses of radiation fail to have any effect on the life-span of moths, probably because of their very short life expectancy; death results from normal causes before the effects of radiation damage become manifest. The greater resistance of moths to sterilisation may be due in part to the "batch production" of eggs as opposed to serial oögenesis in most Coleoptera. In this way, large numbers of eggs in Lepidoptera may be at a particular physiological stage most resistant to radiation, thus increasing the probability of fertility. *R. dominica* and *L. serricorne* are effectively sterilised by the dose of 16,000 rads, evaluated for the control of grain weevils; slight residual fertility in *P. interpunctella* may result in slow build-up in numbers of this species after a number of generations. A dose level higher than 16,000 rads is required for effective control of *C. cautella*.

CONCLUSIONS

1. The effect of dose on adult emergence varied from one species to another; emergence of adult *R. dominica* was not reduced by doses up to 20,000 rads; emergence of male *C. cautella* was reduced by 50 per cent at 28,400 rads and females at 11,600 rads; emergence of *P. interpunctella* was reduced to about 60–70 per cent by 36,000 rads with no difference in susceptibility between the sexes. Emergence of male and female *L. serricorne* was unaffected by doses up to 36,000 rads. Both sexes of *P. interpunctella* emerged with deformed wings, the amount of deformity increasing with dose.
2. Death of *Rhizopertha* was much prolonged after irradiation compared with other species. Pupae and adults of *R. dominica* were equally susceptible to killing by gamma radiation, with the exception of the threshold doses, 3000 and 4000 rads, respectively. With both stages, a 50 per cent kill was obtained at 7000 rads, and 99·9 per cent kill at 25,000 rads, 9 weeks after irradiation.

* Failure to incorporate glycerine in the rearing medium for *Plodia* resulted in the adults being of subnormal weight; the females were also probably of subnormal fecundity. The results obtained for *Plodia* must therefore be treated with some reserve.

3. Only 50–60 per cent of female *C. cautella* mated at 7000–10,000 rads compared with 70 per cent at 20,000 rads and 97 per cent in the control. The number of spermatophores per mated female was significantly reduced at 10,000 rads. Changes in the proportion of mated females and the number of inseminated spermatophores may be attributed indirectly to an effect of irradiation on life-span. The number of mated females of *P. interpunctella* was unaffected by doses up to 36,000 rads. The same dose range increased the number of inseminated spermatophores from 1·74 to 2·83.
4. The two species of beetle were more susceptible to radiation sterilisation than the moths. Pupae and adults of *R. dominica* were equally susceptible to radiation sterilisation; progeny were reduced by 50 per cent and 99·9 per cent at 2200 and 11,000 rads, respectively. Progeny of *C. cautella* were reduced by 50 per cent at 2700 rads and by 99 per cent at 23,500 rads. Progeny of *P. interpunctella* averaged 0·3 per cent of the control at doses from 16,000 to 36,000 rads. The corresponding values for progeny of *L. serricorne* was 0·01 per cent.
5. *R. dominica* and *L. serricorne* are effectively sterilised by 16,000 rads. Slight residual fertility is apparent in *P. interpunctella*. *C. cautella* is incompletely sterilised, and 16,000 rads is unlikely to be effective for its control.

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PAPER XII

A Comparison of the Susceptibility of the Grain Weevil *Sitophilus granarius* (L.) to Accelerated Electrons and ^{60}Co Gamma Radiation

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No difference could be detected in the numbers of emerged adults, rate of emergence or stage at death in the grain when two types of infested wheat, containing mostly pupae, were treated with gamma radiation and accelerated electrons. Mature adults were more susceptible to killing and sterilisation by gamma irradiation. Possible explanations of the observed results are discussed. There is little likelihood of grain weevils providing a useful biological dosimeter in engineering studies for radiation disinfestation of grain.

INTRODUCTION

Many of the more recent studies, in which insects infesting stored products have been tested for susceptibility to ionising radiations, have been carried out with gamma radiation from ^{60}Co.[1–5] In particular, a great deal of information has been accumulated on the effects of ^{60}Co gamma radiation on the grain weevil, *Sitophilus granarius*.[6–9] A large number of studies are also reported on the susceptibility of insects to accelerated electrons,[10–12] but few have set out to compare the effects of these two types of ionising radiations under standardised conditions.[13]

Gamma irradiation and treatment with accelerated electrons are both recognised as potential methods for the commercial disinfestation of stored products. Appraising the two methods for the treatment of grain, Cornwell and Bull[14] conclude that three principal factors discourage commercial exploitation of gamma irradiation: (1) Megacurie sources of ^{60}Co are required to treat grain at handling rates of 200 ton/hr frequently encountered in terminal storage, (2) capital expenditure for these massive sources is high, and (3) large annual throughputs are required to make the estimated operating costs of gamma irradiation competitive with chemical methods.

The recent development of electron machines of high power output and low beam energy[15,16] suggests that treatment with accelerated electrons may be more attractive: machines are available which can treat grain at 200 ton/hr; capital expenditure is lower than for isotope sources and estimated operating costs are highly competitive with existing insect control measures.

Estimates of cost of the two types of irradiation have been based on 16,000 rads (≡ 16,500 rep), the minimum dose for effective sterilisation with ^{60}Co gammas of massive populations of grain weevils of laboratory and wild strains.[6,17] Whilst there is little evidence to suggest major differences in the biological damage caused by the two types of ionising radiation, a comparative study on insect susceptibility is a pre-requisite of development studies with electron machines.

In practice, the efficient use of electrons requires a uniform distribution of dose to moving grain. Evaluation of the best methods of handling cereals for electron irradiation could be facilitated by the use of very small dosimeters which behave identically with the moving insects and moving grains. A stage of the insect itself may afford the ideal dosimeter if marked differences in response are obtained with small increments of dose.

The experiments reported here were carried out with the grain weevil to compare the susceptibility of pupae reared on two types of wheat, and adults reared on one of these varieties, to electron and gamma radiation:

(1) These stages are the most resistant to gamma radiation.
(2) Effects on the insect in grain and the free-living stage can be compared.
(3) Two types of wheat may reveal problems of electron penetration.
(4) Pupae and adults may possess some of the characteristics required of a biological dosimeter.

EXPERIMENTAL

Two experiments were carried out with pupae of five ages, obtained by placing 30,000 adults to oviposit for 24 hr on 8 kg each of soft English (Cappelle) and harder Canadian (Manitoba No. 2) wheat in paper sacks. The wheats were incubated at 26 °C and 70 per cent r.h. and two 150 g samples of each age group and wheat, were irradiated at each of 11 doses. Duplicate untreated samples were retained as controls. The ages of pupae in the first experiment ranged from 26–27 days to 30–31 days after oviposition, and the doses from 10,000–20,000 rads; in the second experiment, pupal ages ranged from 27 to 28 days to 31–32 days after oviposition and the doses from 6000 to 16,000 rads. Small additional samples were taken for dissection to determine stages present at the time of irradiation.

One experiment was carried out with adults obtained by placing 1000 insects to oviposit on 7 kg of grain (Manitoba No. 2) for 3 weeks. They were

0–21 days old at irradiation and treated in batches of 600 at each of 13 doses between 490 and 11,070 rads, with a further sample retained as control.

A comparison of susceptibility to different types of ionising radiation should, ideally, be carried out at the same dose rate (see Jefferies and Banham[18]). This was not possible in the present study, facilities available being used to provide the lowest possible flux of electrons and the highest possible flux of gamma radiation. Electron irradiations were carried out with a 4 MeV linear accelerator, giving 4 rads per pulse each lasting 2·5 micro-seconds, the pulses separated by 1660 micro-seconds. The intensity of radiation was reduced to a minimum (a mean dose rate of $8{\cdot}64 \times 10^6$ rads/hr) by passing the electrons through copper foil 0·014 in. thick. This had additional advantages of spreading the beam and increasing the irradiation area, lowering the peak energy to about 2·5 MeV (closer to the emission energies of ^{60}Co), and reducing the depth for build-up to maximum energy deposition in the test material. Doses were given with an accuracy of ± 5 per cent. Pupae were irradiated in grain in a single layer. Adults were treated in darkness to induce quiescence. Gamma radiation was provided by sources of ^{60}Co, the grain containing pupae being rotated during treatment at a dose rate of $7{\cdot}5 \times 10^4$ rads/hr; doses were delivered with an accuracy of ± 4 per cent. Adults were treated with gamma radiation at $2{\cdot}5 \times 10^5$ rads/hr with the same accuracy.

After irradiation, the insects were held at 26 °C and 70 per cent r.h. In both experiments with pupae, one set of samples treated at each dose level was retained in jars and examined at intervals for adult emergence. When emergence was complete, grains treated at selected dose levels were dissected to assess mortality in the developmental stages. The second set was placed in paper sacks with a further 7 kg of the appropriate type of wheat and left undisturbed until the emerged adults were removed after 4 weeks, and the grain examined for progeny 5 weeks later.

In the experiment with adults, three batches of 100 were each retained on 1 kg of grain in jars and examined for survival 8, 10, 12, 14, 16, 21 and 28 days after irradiation and then discarded. Three similar batches were placed in paper sacks each containing 7 kg of grain and examined for survival at 28 days only. Both jars and sacks were examined for progeny 9 weeks after irradiation.

RESULTS

Irradiation of pupae

At 26 °C and 70 per cent r.h. adults begin to emerge from infested grain 35 days after oviposition. Eclosion is thus expected to occur about 9 days after irradiation of pupae 26–27 days old and about 4 days after irradiation of pupae 31–32 days old. The following criteria were used to measure the effects of the various treatments:

1. Number of adults emerged (found live or dead).
2. Rate of adult emergence.
3. Stage at death in the grain.
4. Production of progeny by emerged adults.

Numbers of adults emerged

Because of known differences in the infestation rates of the two types of wheat (Cappelle, 11–12 per cent and Manitoba, 6–7 per cent), analysis of the data for adult emergence was based on numbers of adults, expressed as a percentage of all stages present in the grain at the time of irradiation. The labour involved in dissecting large numbers of grains permitted this test to be carried out only for the control, 10,000, 15,000 and 20,000 rads in the first experiment, and the control, 6000, 10,000 and 16,000 rad levels in the second experiment. The percentages were transformed to angles and examined by analysis of variance. In the first experiment, very few adults emerged in the youngest samples (26–27 days old), and analysis was thus confined to the four older age groups. In the second experiment, all age groups were examined.

Results of both experiments showed no difference in adult emergence between treatment with gammas and electrons; emergence was markedly depressed with increasing dose with both types of radiation (Table 1). There was also no interaction of gammas and electrons with type of wheat, or age of pupae.

To examine the possible use of pupae as biological dosimeters in plant engineering studies for radiation disinfestation of grain, the numbers of

TABLE 1

Emergence of adults from irradiated pupae. Mean values in angles, with standard errors. Retransformed percentages in parenthesis

Treatment	Pupal experiment 1				Pupal experiment 2			
	Means			S.E.	Means			S.E.
		Gamma	Electron			Gamma	Electron	
Radiation × dose (rads) Interaction	Control:	80·8 (97)	77·9 (96)	±1·35	Control:	76·9 (95)	78·4 (96)	±1·69
	10,000:	38·3 (39)	38·0 (38)		6000:	42·4 (45)	37·0 (36)	
	15,000:	27·7 (22)	29·3 (24)		10,000:	30·1 (25)	28·0 (22)	
	20,000:	20·9 (13)	24·9 (18)		16,000:	18·4 (10)	19·8 (12)	
		Gamma	Electron			Gamma	Electron	
Radiation × pupal age (days) Interaction	27–28:	27·0 (21)	29·4 (24)	±1·35	27–28:	28·7 (23)	30·7 (26)	±1·89
	28–29:	32·1 (28)	33·1 (30)		28–29:	32·8 (29)	33·9 (31)	
	29–30:	52·5 (63)	51·8 (62)		29–30:	45·3 (51)	39·7 (41)	
	30–31:	56·0 (69)	55·7 (68)		30–31:	46·9 (53)	45·4 (51)	
					31–32:	56·1 (69)	54·6 (66)	

adults which emerged at each dose level were expressed as percentages of control and subjected to analysis of regression. The data were bulked for type of wheat and radiation. In the first pupal experiment, the slopes and intercepts of the regression lines for the two methods of post-irradiation retention were not significantly different (Fig. 1). In the second experiment, the slopes

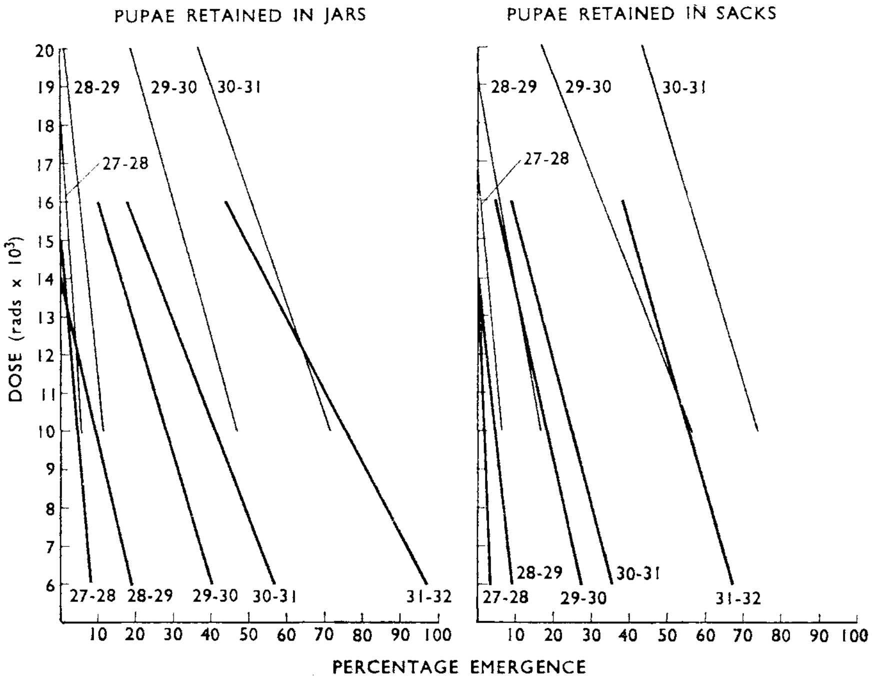


FIG. 1. Relationships between adult emergence and dose in pupal experiment 1 (thin lines) and experiment 2 (thick lines) in relation to age and post-irradiation retention. Correlation coefficients ($P < 1$ per cent or $< 0{\cdot}1$ per cent) range from 0·81 to 0·98.

were markedly different; more frequent examination of samples retained in jars stimulated a considerably higher adult emergence at low doses. There were also marked differences between the two experiments in the response of pupae of the same nominal age.

The usefulness of pupae for dosimetric studies will be maximal if large differences in emergence can be induced by small differences in dose, with preferably a linear relationship between these factors over the dose range being investigated. These requirements were only fulfilled by the oldest samples of pupae (Fig. 1); in the first experiment, emergence from pupae 30–31 days old ranged from 45–75 per cent spanning the dose range 10,000–20,000 rads. In the second experiment, emergence from pupae 31–32 days old ranged from

40–70 per cent in sacks, and from 40–100 per cent in jars, both treatments spanning the range 6000–16,000 rads. Clearly, the response of these age groups affords a more useful measure of dose received than samples less than 30 days old.

Rate of adult emergence

Rate of adult emergence was examined by periodic sieving of samples retained in jars. In the first experiment, counts of adults were obtained at weekly intervals from 1 week after the beginning of emergence, and 80 per cent of adults appeared within that first week. In the second experiment, examinations were brought forward to the day of expected emergence, and to 3 days, 1, 2 and 3 weeks. At 3 days and 1 week, about 20 per cent and 85 per cent, respectively, of the total had emerged. For the purposes of computation, the data of the first experiment were grouped for the two dose ranges, 10,000 to 14,000 rads and 15,000–20,000 rads; in the second, the data were grouped for 6000–10,000 rads and 11,000–16,000 rads. The results for young pupae, 26 to 27 days old, were again excluded.

Only slight differences of up to 5 per cent in rate of emergence were found between the two types of radiation; there was no difference in rate of emergence between the dose ranges 10,000–14,000 rads and 15,000–20,000 rads in the first experiment and 6000–10,000 rads and 11,000–16,000 rads in the second (Table 2). Adults emerged more rapidly, however, from the irradiated samples than from controls – attributable to the emergence period being cut short by the irradiated young insects in the grain failing to emerge as adults.

Stage at death in the grain

Failure of adults to emerge from irradiated infested grains may be due to death of the insects before completion of metamorphosis, or of the pre-formed adults before successful penetration of the testa. If development continues after irradiation, differences in the delayed response period may be demonstrated by the proportion of larvae, pupae and pre-formed adults in grains dissected after all live adults have emerged. The numbers of each stage, recorded at selected dose levels, were calculated as percentages of total infestation, transformed to angles and treated by analysis of variance. In the first experiment (Table 3) there was no difference in the proportion of developmental stages (averaging 12·2 per cent larvae, 9·5 per cent pupae and 24·8 per cent pre-formed adults) in the two types of wheat treated with the two types of ionising radiation. The result of the second experiment was a little less conclusive. English wheat provided a higher proportion of larvae and a lower proportion of pre-formed adults after treatment with gamma radiation than with accelerated electrons. This difference, however, between the two types of radiation was consistent for all age groups, being particularly marked in young samples (27–28 and 28–29 days old), and at all dose levels, including the control. The effect was therefore systematic and must be attri-

TABLE 2

Rate of adult emergence from irradiated pupae. Mean values in angles with standard errors. Retransformed percentages in parenthesis

Dose (rads)	Pupal experiment 1				Pupal experiment 2			
	1 week		2 weeks		3 days		1 week	
	Gamma	Electron	Gamma	Electron	Gamma	Electron	Gamma	Electron
Control	64·2 (81)	62·1 (78)	83·6 (98·8)	83·7 (98·8)	19·3 (11)	23·3 (16)	64·3 (81)	67·9 (86)
6000–10,000	—	—	—	—	27·9 (22)	28·4 (23)	74·2 (93)	73·3 (92)
10,000–14,000	67·8 (86)	64·5 (82)	87·2 (99·8)	85·4 (99·4)	—	—	—	—
11,000–16,000	—	—	—	—	28·3 (23)	29·6 (24)	74·5 (93)	74·1 (92)
15,000–20,000	68·2 (86)	66·8 (84)	86·2 (99·6)	85·5 (99·4)	—	—	—	—
S.E.	±0·75		±0·75		±1·50		±1·12	

TABLE 3

Stage of death in the grain. Mean values in angles with standard errors. Retransformed percentages in parenthesis

Wheat	Pupal experiment 1				Pupal experiment 2			
	English		Manitoba		English		Manitoba	
Radiation	Gamma	Electron	Gamma	Electron	Gamma	Electron	Gamma	Electron
Larvae	20·7 (12)	20·8 (13)	19·9 (12)	20·5 (12)	18·8 (10)	10·9 (4)	12·0 (4)	12·1 (4)
Pupae	18·1 (10)	17·4 (9)	17·5 (9)	18·7 (10)	19·1 (11)	16·1 (7)	18·7 (10)	16·6 (8)
Pre-formed adults	28·0 (22)	29·4 (24)	31·5 (27)	30·6 (26)	27·7 (21)	38·6 (39)	32·3 (29)	35·5 (34)
S.E.	±1·01				±1·40			

buted to experimental technique (probably slight differences in incubation temperature) rather than to a true difference between radiations.

Marked differences were also noticeable between the two experiments in the proportion of developmental stages left in the grain (Fig. 2); they were similar for pupae, but the proportions of larvae and adults were markedly dissimilar. The results indicate that the development of insects which failed to emerge had been much accelerated in the second experiment compared with the first. The cause must be attributed to differences in experimental technique, principally the times of sieving for recording adult emergence. Two points are pertinent: (1) sieving of infested grain stimulates the young adults to emerge, and (2) irradiated adults begin to die about 12 days after irradiation (Cornwell[6,7]). In the first experiment, the first examination for emergence (at 1 week) was 14 days after irradiation of pupae 26–27 days old, 13 days after treatment of those 27–28 days old, and precisely 12 days after treatment of samples aged 28–29 days. Thus, if sieving was carried out before onset of death, larvae may have been induced to moult to pupae and pupae to adults by the stimulation of sieving. In the second experiment, sievings were more frequent, the first on the date of expected emergence, and the second 3 days later. Both these operations occurred within 12 days of the irradiation of all age groups. Thus, if latent radiation damage had not resulted in death before these sievings, continued development to the next stage may have been stimulated.

Progeny production by emerged adults

Examination of the grain in sacks, in which emerged adults from irradiated pupae had been allowed to oviposit undisturbed for 4 weeks, showed that no progeny were produced after treatment with either type of radiation in the dose range 10,000–20,000 rads (Experiment 1). In the second experiment,

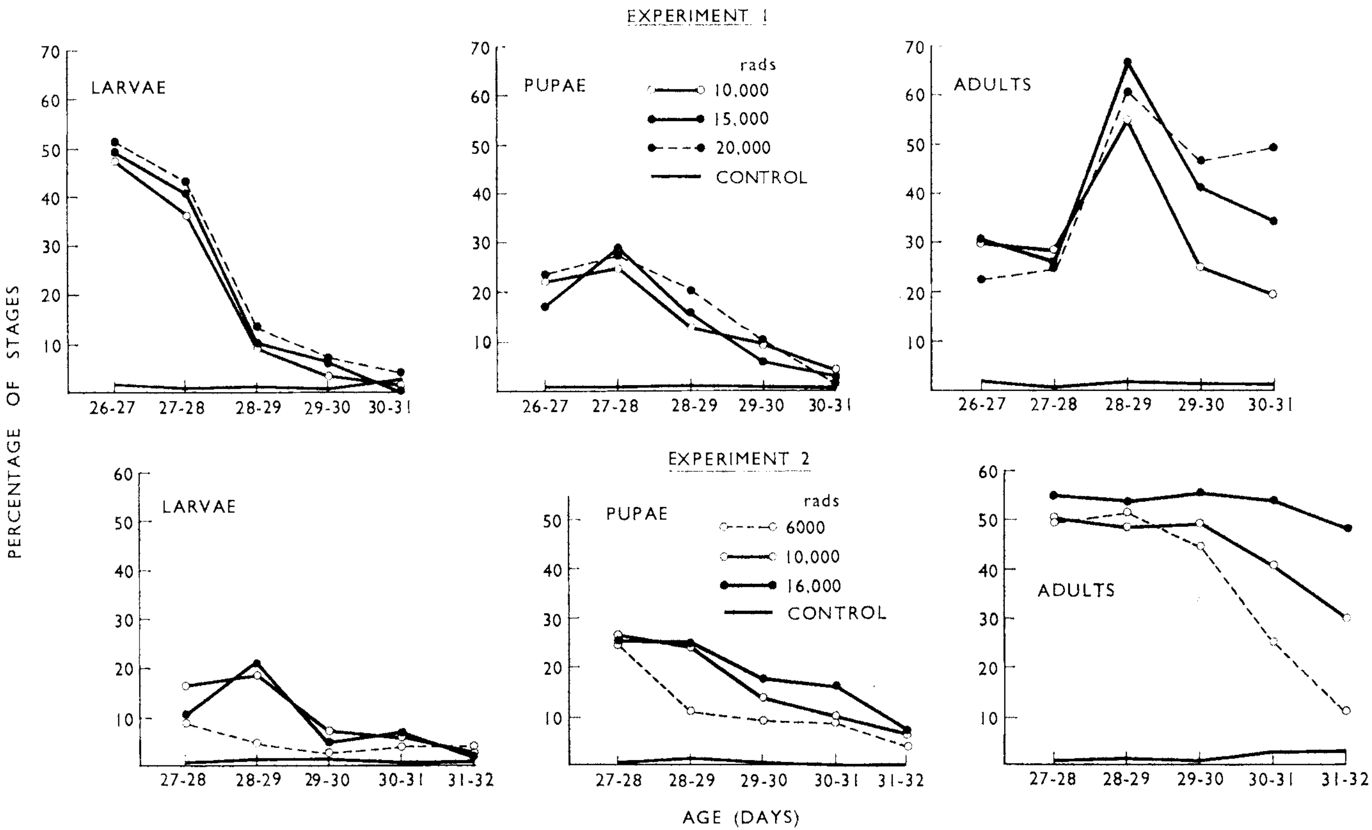


FIG. 2. Percentages of developmental stages left in the grain after adult emergence was complete (Pupal experiments 1 and 2).

very small numbers were produced between 6000 and 10,000 rads, but only by pupae aged 30–31 and 31–32 days at irradiation. The results (Table 4) would suggest that accelerated electrons are less effective than gamma radiation in inducing sterility. Controls on English wheat provided a total of 48,920 progeny compared with 33,560 on Manitoba, again demonstrating the superiority of Cappelle as a rearing medium.

TABLE 4

Progeny production by emerged adults irradiated as pupae (totals for all doses)
Pupal experiment 2

Radiation	Wheat	Pupal age (days)	Progeny	
			Control	All doses
Gamma	English	30–31	13,330	0
		31–32	10,390	21
	Manitoba	30–31	7210	3
		31–32	10,470	6
		Total	41,400	30
Electron	English	30–31	13,280	9
		31–32	11,920	46
	Manitoba	30–31	6710	6
		31–32	9170	134
		Total	41,080	195

Irradiation of adults

Mortality

Twelve days after treatment, about 10 per cent mortality of adults was obtained at doses above 5000 rads with both types of ionising radiation (Fig. 3). Mortality increased markedly during the next 4 days and adults which survived 21 days remained alive until the end of the experiment. There was no difference between radiations in the period of delayed response. At 28 days, the numbers found dead were expressed as a regression of probit mortality upon log dose and the differences in LD 50 for the two types of radiation were highly significant (Table 5), with close agreement between the values for adults retained in jars and sacks. With retention in jars, the estimated dose for 99·9 per cent kill by accelerated electrons (about 13,000 rads) was significantly higher ($P < 0{\cdot}1$ per cent) than by gamma radiation (about 8000 rads). With sack cultures, it was not possible to demonstrate a signi-

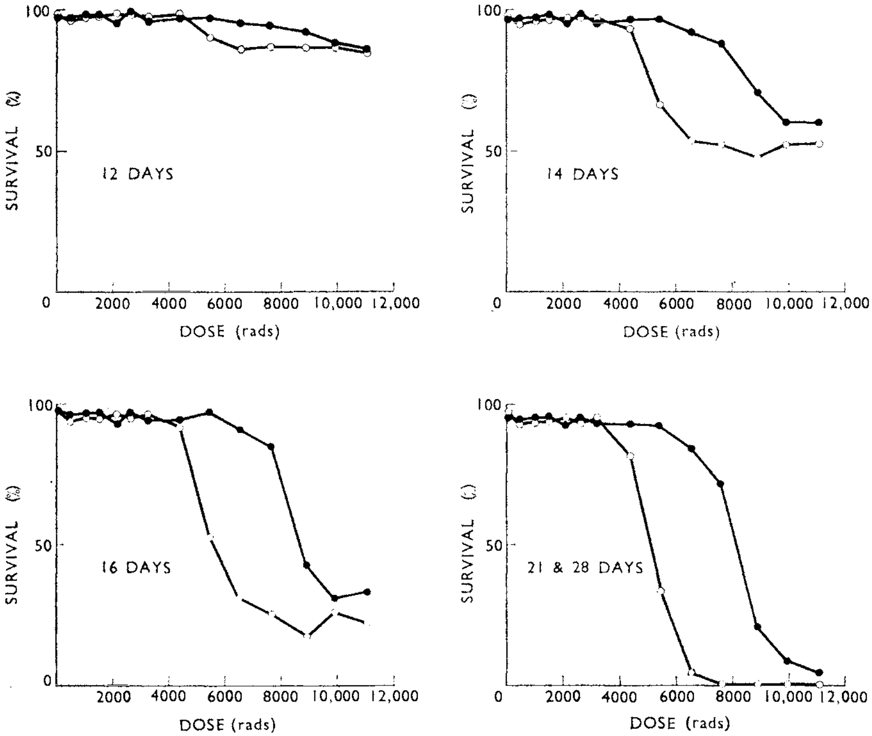


FIG. 3. Survival of adult *S. granarius* at intervals after treatment with either gamma radiation or accelerated electrons. Open circles = gammas; closed circles = electrons. (Data obtained from adults retained in jars.)

ficant difference in the dose for 99·9 per cent kill between electrons and gammas, because of exceptionally high variances. The evidence from this experiment is sufficient, however, to demonstrate that appreciably greater lethality is induced by gamma radiation than with the same dose of accelerated electrons.

Sterility

The number of progeny produced at each dose level was expressed as a percentage of control, and the data then handled for analysis in the same manner as described for mortality, with one exception. It has already been shown[6] that with a wide range of dose levels, transformation of percentage progeny to probits fails to provide a straight-line relationship with log dose. This is because reduction in progeny is caused not only by radiation damage to the reproductive tissues, but also by mortality, and probable disturbances to copulation and oviposition of the survivors. Failure to obtain linearity is particularly marked above the threshold dose for mortality. It does not,

however, militate against the accurate determination of doses for 50 per cent reduction in progeny, since the data is weighted at this point and observed values above and below make a progressively smaller contribution to the estimate. In determining dose levels for 99·9 per cent reduction in progeny it

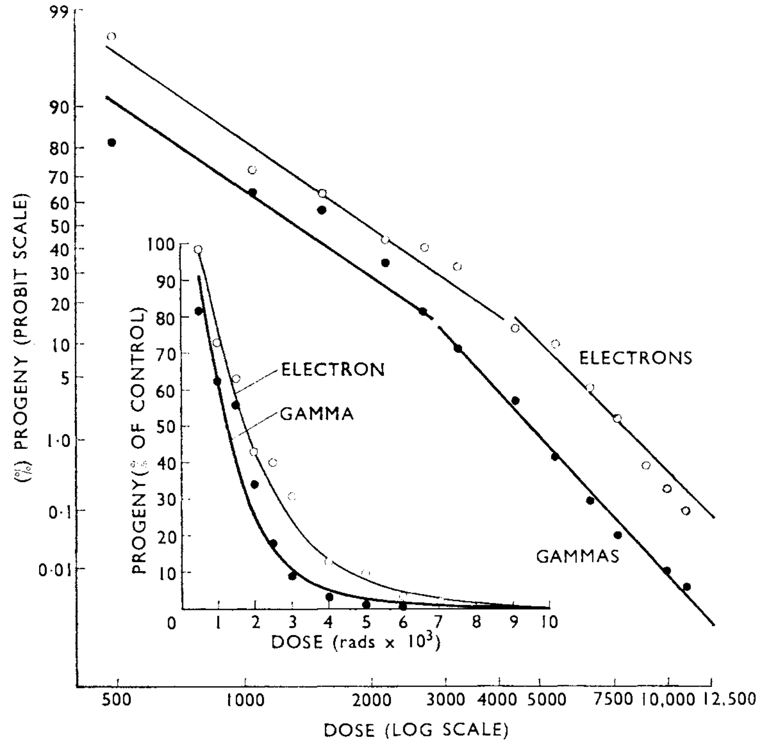


FIG. 4. Probit/log dose relationships for the production of progeny by adult grain weevils treated with electrons and ^{60}Co gammas. Inset shows untransformed relationships.

is necessary to limit observations to those dose levels which give a reduction in progeny to about 10 per cent and less, and in this range the relationship is respectably linear (Fig. 4).

The estimated dose for 50 per cent reduction in progeny (jar and sack cultures) was about 600 rads higher ($P < 0 \cdot 1$ per cent) with accelerated electrons than with gamma radiation (Table 6). There was no difference in the slopes of the regression lines for the two types of radiation. The estimated dose for 99·9 per cent reduction in progeny was 5000 to 8000 rads higher ($P < 1$ per cent and $< 0 \cdot 1$ per cent) for electron radiation than for ^{60}Co gammas. There were also no significant differences in the slopes of the regression lines at the "tail end" of the progeny curve.

TABLE 5

Relationships between dose (gammas and electrons) and mortality of adult grain weevils 28 days after irradiation

Container	Type of radiation	Equation Y (Probit) =	LD 50% Log dose (x) ± S.E.	LD 50% Dose (rads)		LD 99·9% Log dose (x) ± S.E.	LD 99·9% Dose (rads)		S.E. of slope	Probability of significant difference in slope
Jars	Gamma	$16{\cdot}627x - 56{\cdot}73$	$3{\cdot}712 \pm 0{\cdot}004$	5160	$P < 0{\cdot}1\%$	$3{\cdot}898 \pm 0{\cdot}010$	7910	$P < 0{\cdot}1$	$\pm 0{\cdot}957$	Non-sig.
	Electron	$15{\cdot}008x - 53{\cdot}70$	$3{\cdot}911 \pm 0{\cdot}004$	8150		$4{\cdot}117 \pm 0{\cdot}016$	13,090		$\pm 1{\cdot}237$	
Sacks	*Gamma	$8{\cdot}421x - 26{\cdot}31$	$3{\cdot}718 \pm 0{\cdot}009$	5230	$P < 0{\cdot}1\%$	$4{\cdot}085 \pm 0{\cdot}041$	12,170	Non-sig.	$\pm 1{\cdot}013$	Non-sig.
	*Electron	$11{\cdot}537x - 40{\cdot}21$	$3{\cdot}906 \pm 0{\cdot}009$	8060		$4{\cdot}173 \pm 0{\cdot}135$	14,910		$\pm 3{\cdot}476$	

* Not subject to the disturbance of mortality assessments until 28 days.

TABLE 6

Relationships between dose (gamma and electron radiation) and progeny production (28 days oviposition) by irradiated adult grain weevils

Dose for 50 per cent reduction in progeny

Container	Type of radiation	Equation Y (Probit) =	Log dose (x) ± S.E.	Dose (rads)		S.E. of slope	Probability of significant difference in slope
Jars	Gamma Electron	−2·921x + 13·96 −2·779x + 14·03	3·068 ± 0·024 3·250 ± 0·015	1170 1780	$P < 0{\cdot}1\%$	±0·307 ±0·223	Non-significant
Sacks	Gamma Electron	−3·157x + 14·85 −3·184x + 15·47	3·120 ± 0·017 3·290 ± 0·008	1320 1950	$P < 0{\cdot}1\%$	±0·287 ±0·174	Non-significant

Dose for 99·9 per cent reduction in progeny

Container	Type of radiation	Equation Y (Probit) =	Log dose (x) ± S.E.	Dose (rads)		S.E. of slope	Probability of significant difference in slope
Jars	Gamma Electron	−4·147x + 18·22 −3·188x + 15·38	3·934 ± 0·008 4·225 ± 0·074	8590 16,780	$P < 1\%$	±0·101 ±0·456	Non-significant
Sacks	Gamma Electron	−4·975x + 21·12 −4·547x + 20·53	3·862 ± 0·014 4·094 ± 0·038	7270 12,430	$P < 0{\cdot}1\%$	±0·238 ±0·471	Non-significant

Adults retained in jars were subject to the disturbance of mortality assessments after irradiation.

It is clear from these analyses that treatment with electrons is less effective than with ^{60}Co gammas in reducing reproductive capacity. It is unlikely that this difference in degree of sterilisation is influenced indirectly by survival, since Cornwell[(6)] reports that with substerilised adults the major proportion of fertile eggs are laid within the first 2 weeks after irradiation. The current experiments have shown that similar numbers of adults survive up to 12 days after treatment with both types of radiation.

DISCUSSION

The use of grain weevils as radiation dosimeters

Physical, chemical and biological methods are available for measuring doses of ionising radiation. The three principal factors required in the ideal radiation dosimeter are accuracy of dose estimation, repeatability, and speed of determination. It is desirable to distinguish changes in dose distribution, at least at the 5 per cent level of probability. The 95 per cent confidence limits afforded by testing 1300 infested grains containing pupae at each of eleven doses averaged about ± 2500 rads for samples older than 30 days. This level of confidence would be of little value in detecting all but very wide variations in dose. For pupae to be used as dosimeters, confidence limits would need to be reduced by a factor of 10 (± 250 rads); the number of infested grains would therefore need to be increased about 100 times.

Accuracy of dose estimation could be improved by using free-living adults. Their susceptibility does not vary appreciably within a few days[(6)] and known numbers can be used, whereas the numbers of pupae must be estimated from a sample.

Doses for 50 per cent kill, 28 days after irradiation with electrons and ^{60}Co gammas, were obtained by testing only 300 adults at each of 13 doses, and the 95 per cent confidence limits averaged ± 200 rads. The mortality of adults, however, would only be useful as a measure of radiation dose in the range over which the mortality response is reasonably linear, viz. 4500–6500 rads for gamma irradiation and 7500–9000 rads for electrons.

These studies have also demonstrated the difficulty in duplicate experiments of obtaining the same biological response with pupae of the same nominal age. Adult emergence is very much affected by pupal age, even within limits of half a day, and rigid control would obviously be necessary in incubating the grain to obtain pupae. The marked difference in response with frequency of handling (jars vs. sacks) indicates that extreme care would also be necessary with respect to disturbance.

To determine the dose received, both pupae and adults would need to be separated from the bulk by sieving, which may adversely affect mortality. A lapse of 3 weeks after irradiation is the minimum necessary to obtain results

with both pupae and adults, and this would impose considerable delay in the interpretation of changes in plant design.

At present, development of calorimetric methods has not reached a stage which permits the use of physical dosimeters in studies of moving grain. Ferrous sulphate provides one of the most useful chemical dosimeters for measuring doses in the range 10,000–20,000 rads; doses can be measured with an accuracy of ± 2 per cent, results are repeatable under well-controlled conditions and evaluations can be made very rapidly. Successful attempts have been made at this laboratory (Whittaker, unpublished) to produce small plastic sachets of ferrous sulphate comparable in size and weight with individual grains. Whilst they have not been tested in moving grain, this type of chemical dosimeter offers a distinct advantage over biological methods, viz. one dosimeter can provide an estimate of dose whereas many pupae or adults of the grain weevil are required.

Susceptibility to accelerated electrons and ^{60}Co gammas

Investigations into the effects of ionising radiation on the grain weevil have been made by a number of previous workers. Comparison of the results obtained in independent studies with electrons and gammas is of doubtful value because of differences in experimental technique and the frequent use of high doses (often in excess of 100,000 rads) which mask small differences in relative biological efficiency.

The present work could detect no difference between the two types of radiation on pupae with respect to emergence of adults, rate of emergence and stage at death in the grain, but adults obtained from irradiated pupae produced more progeny after treatment with electrons than with gamma radiation. Marked differences, however, were found with the irradiation of adults; a higher dose was required to reduce survival and fertility to 50 per cent and 99·9 per cent with electrons than with ^{60}Co gammas.

The difference in response to the two radiations was not anticipated; indeed, it was thought that electrons would be equally, or even more, effective at the high dose rates employed. In studies with gamma radiation into the effects of dose rate on adults of *T. confusum* and *O. surinamensis*,[18] treatment at 4000 rads/hr was more lethal than at 2000 rads/hr. Chronic treatment has also been shown by many workers to be less effective than acute irradiation.

There are three possible explanations for the results of the present work:

(1) Errors in dosimetry, or dose received by the insect.

(2) A different biological effectiveness of pulsed (electron) and continuous (gamma) radiations.

(3) An effect of dose rate operating through oxygen tension.

Errors in dosimetry can arise if the build-up of energy deposition at tissue surfaces is ignored. With ^{60}Co gammas, electron equilibrium occurred within the walls of the sample containers. The thickness for build-up for 4 MeV

electrons passing through water is 4–5 mm, but the use of copper foil in the 4 MeV beam, and the passage of the beam through a metre of air would reduce the build-up distance considerably. Dosimetry of the electron beam was carried out with ferrous sulphate in flat plastic sachets with a mean thickness of 3 mm, equal to the mean thickness of the experimental grains. Electron equilibrium thus occurred both in the ferrous sulphate and irradiated wheat and ensured that pupae received an accurate dose of radiation. Adults, however, which are only about 1 mm thick, and were freely exposed to the electrons, may have received slightly smaller levels of radiation than measured by the dosimeters. Calculations based on data provided by Hine and Brownell[19] suggest that with mono-energetic 2·5 MeV electrons the maximum error in dose received would not exceed 15 per cent. Since a copper foil was used to produce a scattered electron beam, the error in dose would again be much less. The results, however, show that at the 50 per cent level for mortality and sterility, the dose of electrons to produce the same response as with gammas was about 50 per cent higher.

Jefferies[9] has shown that when doses of gamma radiation are split into several fractions, interrupted treatments are less effective than continuous. He could not detect differences, however, when intervals between doses were less than 10 min. Using the protein β-lactoglobulin, Glew *et al.*[20] also found no change in protein denaturation or $-SH$ concentration when the pulse rate of electrons was varied between 5 and 600 pulses per sec. It seems most unlikely, therefore, that the differences observed in the present work can be attributed to "recovery" from radiation damage between pulses separated by only 1·7 milliseconds.

It has already been emphasised that studies of relative biological efficiency should ideally be carried out at the same dose rate. In the present work, the peak dose rate of the electrons ($5{\cdot}76 \times 10^9$ rads/hr) was about 80,000 times greater than the highest flux of gamma radiation ($2{\cdot}5 \times 10^5$ rads/hr). During irradiation, oxygen is removed in the formation of peroxides. At very high dose rates, oxygen is depleted rapidly and tissues may therefore be irradiated under intracellular anoxia; radical recombination may also reduce the number of free radicals available for the production of peroxides. That the resistance of *S. granarius* can be noticeably increased by irradiation under conditions of depleted oxygen has already been observed.[3,17] It follows from this evidence that radiation susceptibility will be determined by the oxygen balance between depletion and replenishment by diffusion and respiration. In studies of the respiratory behaviour of the developmental stages of the rice weevil *(S. oryzae)* and the lesser grain borer *(R. dominica)*, Birch[21] states that oxygen consumption increases to a maximum during the last larval stage (prepupa), falls during pupation and rises again as the adult matures. It seems probable, therefore, that dose rate offers the most plausible explanation for the difference in effectiveness of the two radiations.

The results of this investigation suggest that 16,000 rads, the minimum

dose for effective sterilisation of *S. granarius* with gamma radiation, may not give the same degree of control with electrons delivered at a high dose rate. With ^{60}Co gammas, a 99·9 per cent reduction in progeny was obtained under ideal conditions (sacks) with 7300 rads, and with accelerated electrons at 12,400 rads (Table 6). As the relationships between sterility and dose were of similar slope for both types of radiation, the estimated dose for complete sterilisation by electrons may be estimated as $\frac{16,000}{7,300} \times 12,400$, i.e. about 27,000 rads. This estimate, however, cannot be regarded as accurate since possible errors are acknowledged in the dose delivered to the insects by electrons. It would therefore seem desirable that the results of this investigation be accepted with some reserve and that further experiments be carried out with adults to examine the efficacy of accelerated electrons at 16,000 rads.

CONCLUSIONS

1. Experiments were carried out with the grain weevil *(S. granarius)* to compare the susceptibility of pupae in English and Manitoba wheats, and mature adults, reared on Manitoba only, to accelerated electrons and ^{60}Co gamma radiation. An assessment was also made of the usefulness of pupae and adults of the grain weevil as radiation dosimeters in engineering studies for radiation disinfestation of grain.
2. No difference was found between the two radiations on pupae with respect to emergence of adults, rate of emergence, or stage at death in the grain with either type of wheat. Accelerated electrons were less effective than gamma radiation in inducing sterility in adults obtained from irradiated pupae.
3. Mature adults were more susceptible to the killing and sterilising effects of gamma radiation. The estimated dose for 50 per cent kill was about 3000 rads higher for accelerated electrons than with ^{60}Co gammas: the estimated dose for 50 per cent reduction in progeny was about 600 rads higher. The difference in dose for 99·9 per cent mortality and sterility with the two radiations was about 5000 rads.
4. The greater resistance of adults to accelerated electrons may be attributed partly to failure in ensuring electron equilibrium within the insect during irradiation, and partly to the rapid depletion of oxygen at a high dose rate, resulting in treatment under intracellular anoxia. The level of sterility obtained by treatment of adult grain weevils with 16,000 rads of accelerated electrons (minimum effective dose with ^{60}Co gammas) requires further investigation.
5. Adults provide more accurate estimates of dose than pupae when used as radiation dosimeters. Both stages are likely to be less useful in engineering studies than chemical dosimeters.

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PAPER XIII

The Effect of Dose Rate on the Response of *Tribolium confusum* Duv., *Oryzaephilus surinamensis* (L.) and *Sitophilus granarius* (L.) to ^{60}Co Gamma Radiation

D. J. JEFFERIES and E. J. BANHAM

Modifications were obtained in the response of three stored-products pests, *Tribolium confusum*, *Oryzaephilus surinamensis* and *Sitophilus granarius*, to variations in dose rate of gamma radiation from 1500–4700 rads/hr. The results for the grain weevil, compared with previous observations at much higher dose rates, suggest a parabolic relationship between dose rate and LD 50 (or SD 50), affording an optimum radiation intensity for the disinfestation of grain.

INTRODUCTION

In tests of the efficacy of conventional insecticides, fumigants or contact poisons, it is usual to equate dosage with concentration of the chemical, or with the time of exposure. Under conditions where treatment may continue over an extended period, such as during fumigation, dosage is equated with a function of the two, namely, the concentration × time product. This simple relationship, however, may be only an approximation if the poison is in low concentration and has to penetrate to a vital centre within the organism, if there is a threshold concentration which produces no toxic effect, or if there are active mechanisms of detoxification.[1]

In tests of ionising radiations, for their killing or sterilising properties on insects, the intensity of treatment, dose rate, has not merited particular attention, although in biological experiments as a whole, dose-rate effects are common and indicate that restoration and repair processes may occur.[2] In previous experiments with the flour beetle, *T. confusum*, treated by chronic irradiation,[3,4] competition between injury and restoration resulted in an extension of adult life span, some surviving twice the dose that produced death by acute irradiation. Again, in chemical reactions which require oxygen to diffuse into a system, dose-rate dependence has also been demonstrated; oxygen imbalance between depletion and replenishment was thought[5] to be the cause of reduced biological damage in adult weevils treated with high

dose rate electrons, treatment under anoxia having been shown to be less effective than in air.[6,7]

The experiments reported here were carried out to assess the need for close control of dose rate in laboratory evaluations of ionising radiation for disinfestation. Tests are reported on variations in response of three stored products pests, the confused flour beetle *(T. confusum)*, the saw-toothed grain beetle *(O. surinamensis)* and the grain weevil *(S. granarius)* to dose rates varying from 1500 to 4700 rads/hr.

EXPERIMENTAL

All irradiations were carried out in a ^{60}Co source,[8] with *T. confusum* and *O. surinamensis* at 30 °C and *S. granarius* at 26 °C. Two types of treatment were investigated: (1) doses delivered to samples placed at the same distance from the source and the time of exposure varied, viz. at a constant dose rate, (2) doses delivered during the same period to samples at varied distances from the source, viz. given at different dose rates. The first type of treatment necessitates interrupting irradiation to remove samples as they accumulate the required dose; breaks were of less than 1 min, as longer periods have been found to modify the lethal response[9] at least of *S. granarius*.

Samples of adult *T. confusum* and *O. surinamensis* were 0–14 days old at irradiation and those of *S. granarius* were 0–21 days old. Batches of 50 were used at each dose, contained in small ventilated polythene tubes on 3 g of culture medium; those of the flour beetle were irradiated on wholemeal flour, the saw-toothed beetles on cracked wheat and the grain weevils on whole wheat. After irradiation each batch was held on 100 g of its appropriate food at 30 °C and 60 per cent r.h., with the exception of *S. granarius*, for which pairs of samples, treated at the same dose, were placed together on 1 kg of wheat and retained at 26 °C and 70 per cent r.h. Mortality of *T. confusum* and *S. granarius* was recorded after a month, but after a fortnight for the shorter-lived *O. surinamensis*. The data, corrected for natural mortality,[10] were examined by Probit analysis. Fertility was calculated from the numbers of progeny per live parent per month for *T. confusum* and *S. granarius* and from the first week of oviposition by *O. surinamensis*; the dose/fertility response curve was treated as an exponential function.

RESULTS

Tribolium confusum

The mean effect on survival, 4 weeks after irradiation of adult flour beetles at constant dose rates of 2000 and 4000 rads/hr and at variable dose rates given in constant exposure periods of 4·75 and 2·375 hr is shown in Fig. 1. Three samples were used in each treatment. Doubling the dose rate from

2000 to 4000 rads/hr reduced survival at all doses; a similar result was obtained by halving the exposure period. Examination of the data by Probit analysis (Table 1) showed a significant reduction in LD 50 ($P < 0{\cdot}1$ per cent) from 9330 rads for treatment at 2000 rads/hr to 8290 rads at 4000 rads/hr.

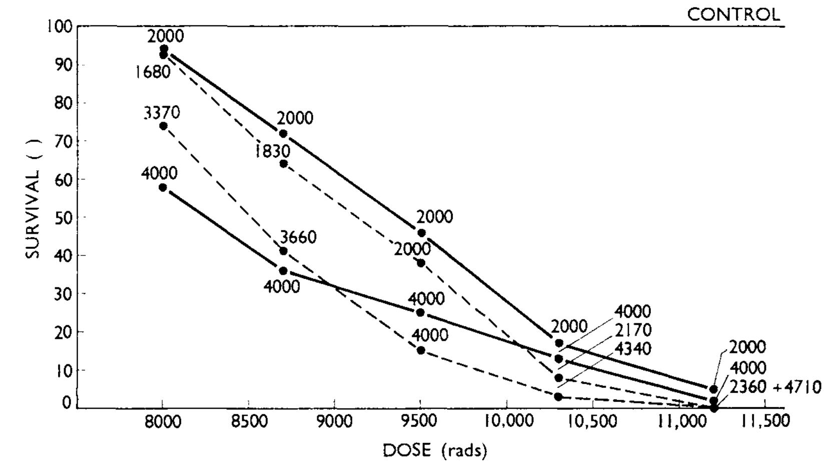


FIG. 1. Survival of adult *T. confusum*, 28 days after irradiation at constant dose rates (2000 and 4000 rads/hr—continuous lines) and constant exposure periods (2·375 and 4·75 hr—broken lines). Dose rates for each treatment are shown.

There was a significant, but less marked reduction in LD 50 ($P < 1$ per cent) from 9090 rads with a constant irradiation exposure of 4·75 hr (variable dose rate 1680–2360 rads/hr) to 8530 rads with an exposure of 2·375 hr (variable dose rate 3370–4710 rads/hr). In both tests, with constant dose rate and constant exposure period, treatments at 9500 rads were given at 2000 rads and 4000 rads/hr: the dose/response curves would thus be expected to intersect. This did not occur (Fig. 1), but analyses showed no statistical difference in response (4000 rads/hr and 2·375 hr, $P > 10$ per cent; 2000 rads/hr and 4·75 hr, $P > 5$ per cent). The doses used in this experiment were almost completely sterilising and no comparison between treatments was made for progeny production.

Oryzaephilus surinamensis

Two experiments were carried out with *O. surinamensis*, one to examine the effect of dose rate on survival in the dose range 8250–20,000 rads, the other to examine the effect on fertility between 1440 and 7000 rads. In both experiments, a comparison was made between treatment at a constant dose rate and treatment with a constant exposure period. Five samples were used at each dose.

TABLE 1

50 per cent lethal and sterilising doses for T. confusum, O. surinamensis *and* S. granarius *irradiated at constant dose rates and with constant exposure periods (varying dose rates)*

Survival					Fertility
Species	Dose rate (rads/hr)	Time after irradiation (days)	Dose for 50% reduction in survival		Dose for 50% reduction in progeny per parent ± St. Error (rads)
			Log dose ± St. Error	Dose (rads)	
T. confusum	constant 4000	28	3·919 ± 0·005	8290	
	constant 2000	28	3·970 ± 0·003	9330	
	varying 3370–4710	28	3·931 ± 0·003	8530	
	varying 1680–2360	28	3·959 ± 0·002	9090	
O. surinamensis	constant 4000	14	4·088 ± 0·004	12,250	2310 ± 162
	varying 1650–4000	14	4·127 ± 0·006	13,400	3390 ± 154
S. granarius	constant 4000	28	3·781 ± 0·010	6040	1990 ± 182
	varying 1500–4000	28	3·818 ± 0·003	6570	1600 ± 242

The mean effect on survival, 2 weeks after irradiation at 4000 rads/hr and at a variable dose rate (1650–4000 rads/hr) given in a constant period of 5 hr (Fig. 2), again indicates greater radiation damage with the more acute treatment. Table 1 shows a significant reduction in LD 50 from 13,400 to

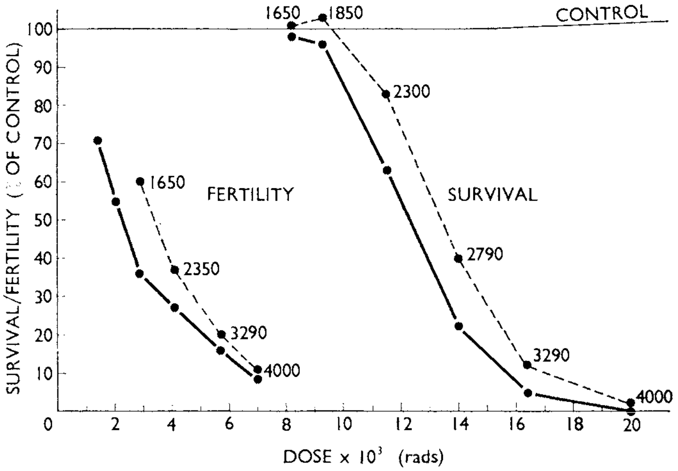


FIG. 2. Survival of adult *O. surinamensis*, 14 days after irradiation, and fertility (progeny/parent during first week of oviposition) with treatment at a constant dose rate (4000 rads/hr— continuous lines) and with constant exposure periods (survival, 5 hr; fertility, 1·75 hr— broken lines). Dose rates for each treatment are shown.

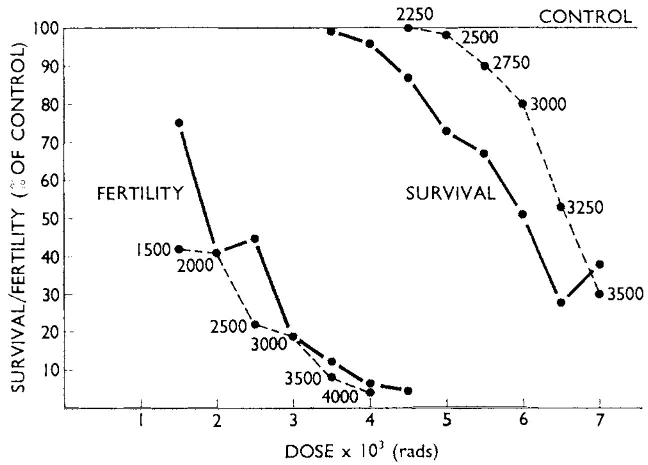


FIG. 3. Survival of adult *S. granarius*, 28 days after irradiation, and fertility (progeny/parent during first month of oviposition) with treatment at a constant dose rate (4000 rads/hr—continuous lines) and with constant exposure periods (survival, 2 hr; fertility, 1 hr— broken lines). Dose rates for each treatment are shown.

12,250 rads ($P < 0{\cdot}1$ per cent) for the two treatments. There was also a significant drop ($P < 1$ per cent) in dose for a 50 per cent loss in fertility, namely from 3390 rads for treatment (1650–4000 rads/hr) given in a constant exposure period of 1·75 hr to 2310 rads given at a constant 4000 rads/hr.

Sitophilus granarius

In a similar experiment with the grain weevil, a comparison was made between survival of adults treated in the range 3500 to 7000 rads at a constant dose rate of 4000 rads/hr and at a variable dose rate (2250–3500 rads/hr) given in a constant period of 2 hr. The test for fertility was carried out in the lower dose range, 1500–4500 rads, to compare treatment at 4000 rads/hr with a variable rate (1500–4000 rads/hr) given in 1 hr. Four samples were used at each dose.

The mean effect on survival, 4 weeks after irradiation (Fig. 3), further substantiated the greater lethality with the more acute treatment; the LD 50 (Table 1) was significantly reduced from 6570 to 6040 rads ($P < 1$ per cent). The SD 50's, however, were not significantly different.

DISCUSSION

These experiments have shown that dose rate, within the range 1500 to 4700 rads/hr, modifies the lethal response of three insect pests of stored products to gamma irradiation; high dose rates induce greater mortality and provide a lower LD 50. This modification in response is an expression of recovery from radiation damage to an extent allowed by the period of radiation exposure; acute treatment involves short exposure periods such that the period for recovery may be negligible. Support to this suggestion, as an explanation of the observed results, is given by the earlier demonstration by Jefferies[11] of restoration occurring in the grain weevil during and after irradiation. Competition between injury and restoration at low dose rates precludes an expression of radiation damage; no suppression of mitosis occurred in the pollen of *Tradescantia* irradiated over long periods[12] and no change took place in populations of the insects *T. confusum*[3,4] and *Drosophila*[13] under chronic irradiation. The evidence suggests that a lethal response dependent upon dose rate may be common to all insect species, but exceptions have been observed; larvae of the powder post beetle *(Lyctus brunneus)*[14] emerged from eggs treated at 6000 r but not with 8000 r, whether irradiated at 50 or 1085 r/min – a difference in radiation intensity of about 20. This particular result, however, may be attributed to the choice of doses which could mask a dependence of lethality upon dose rate.

Using *O. surinamensis*, the present work has also demonstrated an effect

of radiation intensity on insect sterility. Grosch and Sullivan[15] (Grosch, too, in personal communication to Howden[16]) and Rhode *et al.*[17] have also shown an increased effect of radiation with intensity on *Habrobracon juglandis* and *Anastrepha ludens*, respectively. Both show that fecundity as well as fertility is dependent upon dose rate. It is surprising, therefore, that no significant effect of radiation intensity was obtained in the present work with *S. granarius*. Using the same species of weevil, Mortreuil[18] also found no modification in sterility at 8000 r with treatment given at 50, 500 and 1375 r/min. This confirmation, however, is of little value since 8000 r itself is almost completely sterilising.[19] Of greater significance is the observation of Jefferies[11] that dose fractionation with intervals of 1 day also allowed no recovery of fertility in *S. granarius*, the effect on the germ cells being apparently cumulative. With intervals of 2 days, dose fractionation did modify the response, suggesting that recovery in reproductive integrity is so slow in this insect as to indicate no dependence upon dose rate, at least in the narrow range investigated here. The maturation rate within the gonads of this species may be slower than in those mentioned above. With a 62-fold increase in dose rate (4000 rads/hr in the present work compared with 250,000 rads/hr used by Bull and Cornwell,[5]) the SD 50 was significantly reduced ($P < 0{\cdot}1$ per cent) from 1990 rads (Table 1) to 1120 $\pm$ 90 rads.*

Although many results are available from studies with *S. granarius* at this laboratory, only those in which insects were reared and retained under identical conditions[20] are valid for a comparison of the effects of dose rate. Again using the data of Bull and Cornwell[5] (which fulfil this requirement) the dose for 50 per cent kill of *S. granarius* at 250,000 rads/hr was 5160 rads (log LD 50, $3{\cdot}712 \pm 0{\cdot}004$), again significantly lower ($P < 0{\cdot}1$ per cent) than 6040 rads (Table 1) obtained at 4000 rads/hr in the present work. In this instance the difference in LD 50 – of about 15 per cent – corresponds to a 62-fold increase in dose rate. The disparity in LD 50 obtained in the present work with only a two-fold difference in dose rate (constant 4000 rads/hr compared with varying 1500–4000 rads/hr – a mean of about 2800 rads/hr) was about 8 per cent. It would thus appear that lethality may be modified by dose rates well above 4000 rads/hr but not to the same extent as below 4000 rads. There is in fact evidence to suggest[5] that at very high dose rates ($8{\cdot}64 \times 10^6$ rads/hr) which may be achieved with accelerated electrons, there is an increase rather than a decrease in the LD 50 over that obtained with 250,000 rads/hr of ^{60}Co gammas. This has been attributed to oxygen depletion within the tissues resulting in irradiation under intracellular anoxia. Similarly, the SD 50 at this high radiation intensity may also be modified by oxidative reactions; the relationship between susceptibility (LD 50 or SD 50) and dose rate would thus appear to be a parabolic function, with an optimum

* For the purpose of this comparison the data of Bull and Cornwell was re-calculated in terms of progeny per parent.

dose rate for mitotic and meiotic derangement, and thus for a commercial process of radiation disinfestation of grain, possibly in the range 10^4–10^6 rads/hr.

Clearly, this study shows that the concept of a constant response to a concentration × time product as applied to fumigation does not hold for insect control by irradiation. Recovery to radiation damage does, however, compare with mechanisms of chemical detoxification. Certainly in laboratory irradiation studies, a constant dose rate should be used in evaluating dose/response relationships; the dose rate should also be stated in reporting the investigation.

CONCLUSIONS

1. The lethal response of three stored products pests, *T. confusum*, *O. surinamensis* and *S. granarius*, to gamma radiation is modified by variations in dose rate in the range 1500–4700 rads/hr; the higher the dose rate the greater the mortality and the lower the LD 50. A similar response to radiation intensity is obtained in the level of sterility of *O. surinamensis*; no change in the level of sterility of *S. granarius* occurs within the range investigated.
2. Comparison of the results with previous observations on *S. granarius*, irradiated at much higher dose rates, suggests a possible parabolic relationship between dose rate and LD 50 (or SD 50), modifications at low radiation intensities being attributable to processes of recovery and at high radiation intensities to depletion of oxygen. An optimum intensity for commercial disinfestation of grain by irradiation is suggested.
3. The effect of dose rate is particularly marked at low radiation intensities normally used in laboratory investigations; to avoid modification of dose/response curves, the use of constant dose rates is preferable to constant time exposures.

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Editor's Discussion

THIS discussion identifies the problems which may arise in the application of radiation disinfestation of grain as an industrial process. Grain handling procedures are examined together with the design, cost and development of the irradiation process. The effects of irradiation on grain in respect of wholesomeness, manufacturing properties and taste are discussed and an appraisal made of the entomological findings with suggestions for future research.

Summing up at the conference of the United Nations' Food and Agriculture Organisation (Harwell, 1958) on the "use of ionising radiations for food preservation", the meeting believed that among applications requiring low dose treatment, disinfestation of grain was approaching the stage at which commercial exploitation might be considered. Entomological research and studies on the acceptability and wholesomeness of irradiated grain, carried out over the following 5 years, substantiate this view. Technical feasibility, however, is not enough to ensure ultimate application. To be acceptable any *new* process must offer distinct advantages over conventional methods; it must be more effective, more easily applied and competitive in price. Only by a thorough understanding of existing trade practices will it be possible to assess the value of irradiation; only by ensuring that industries, which may ultimately accept the process are aware of the possible advantages or limitations of this new control method is it possible to obtain some expression of the demand for radiation treatment. It is unfortunate that research into the potential uses of radiation for industrial processing has often lost sight of the fact that application must be compatible with commercial interests. With this in mind, the studies at Wantage were discussed at various stages of progress with manufacturers of grain handling equipment, bodies responsible for infestation control, grain silo managers and cereal chemists, well aware of the qualities required in cereals for processing and manufacture. It is also for this reason that the entomological papers reported here do not embrace studies in fundamental radiobiology to investigate radiation effects at the tissue and cellular level.

It is also unfortunate that in the early 1950's, the potential of an irradiation process for the treatment of stored foodstuffs was appraised against a background of "enormous annual losses caused by insects, the upward trend in world population, the starving millions and the need to safeguard crops once harvested". It is still irrational to place irradiation before known, proven pest

control procedures as the most expedient way to conserve world food supplies to accommodate an ever-increasing population. Certainly from a study of trade practices in Australia, it is clear that the benefits of radiation disinfestation are divorced from the issues of world under-nourishment and the distribution of surpluses. The process must be considered as a commercial proposition and not as a benevolence to mankind.

GRAIN STORAGE AND HANDLING

The principal requirements for the storage and handling of cereals in the large producing countries are:

1. Adequate capacity and distribution of storage facilities in the up-country production areas to accommodate and retain each season's harvest in good condition for variable periods not usually exceeding 12 months.
2. An efficient transport network (road, rail or water) from up-country storage to terminal facilities to meet the requirements of domestic and export markets.
3. Adequate handling and storage facilities at terminal elevators for the rapid intake of grain and its discharge, principally to shipping.
4. In some countries, specially designed facilities, strategically placed, for the retention of surplus wheat or national reserves.

Using Australia as an example, about 800 up-country storages hold grain in bags and bulk, some as far as 400 miles from the ports. Storage facilities at the port elevators consist of vertical concrete bins, or horizontal storages in which bulk grain is piled on the floor. The capacity of storage varies from 1 million bushels to over 22 million (600,000 tons) at Geelong – Australia's largest terminal (frontispiece). Usually, elevators handle very much more in a season than their holding capacities; rapid intake, cleaning and weighing, safe storage, and discharge to boats, demand elaborate equipment for conveying the grain horizontally and vertically. The carrying capacity of the equipment is related to the requirements of rapid movement, rather than to the storage capacity of the terminal itself.

Movement of grain at an export terminal

Grain at intake is received from a number of hoppers which feed belt or drag chain conveyors; elevation is by belt and bucket or perhaps pneumatic conveyors if receiving from boats. Distribution to bins or horizontal storage is carried out by overhead belt conveyors and gravity chutes. Grain is stored for varying periods, during which it may be moved from one bin to another to maintain condition ("turning"), or passed to special bins equipped for fumigation. Drag chain conveyors move the grain, totally enclosed, at 90 ft/min at capacities up to 100 ton/hr; belt conveyors may reach speeds of 800 ft/min, and convey grain most frequently at 200–400 ton/hr, and in

pneumatic conveyors the grain is lifted, at up to 200 ton/hr, by air speeds approaching 100 mile/hr. In modern terminal elevators the operation of all equipment is usually by remote control from a central panel, the various operations being shown on a mimic diagram with push-button switches, indicator and warning lights.

In preparation for rapid discharge to ocean-going vessels, special shipping bins may be filled at the terminal a few days before the boat arrives. This is particularly the case if grain is being moved from horizontal storage. Discharge from bins is controlled by valves at the base, the grain falling on to conveyors. Grain may be discharged from a terminal at a number of points and is conveyed, often at 400 ton/hr, along overhead galleries to points directly over the holds. Using two Australian terminals as examples of grain intake and discharge, wheat entering the Sydney terminal (N.S.W.) is moved from rail trucks by five conveyors at about 500 ton/hr, the maximum total carrying capacity being 1000 ton/hr. It is handled within the terminal at 250 ton/hr and discharged to boats through six spouts which can be used simultaneously on large modern vessels. Nowadays shipments of 21,000 tons or more are not uncommon at this terminal; the fastest loading time for a 21,000 ton self-trimming ship is 14 hr, which represents an average discharge rate of 1500 ton/hr. Intake at Geelong (Victoria) proceeds along four belts at 1200 ton/hr and it is discharged at a maximum rate of 1600 ton/hr. In practice the normal loading time for a vessel of 10,000 tons averages 18–20 hr (500–550 ton/hr).

Two case-histories of terminal handling

The primary factor which influences the economics of the radiation process is the annual throughput of grain. This may vary not only from year to year, but during different periods of the year; with the kind permission of the New South Wales Grain Elevators Board, extracts from the records of the Sydney and Newcastle terminals are presented as examples.

Annual intake of wheat at Sydney averaged a quarter of a million tons over the 9-year period 1951–9 (Table I): intake for 1957, a season of prolonged drought, was 70 per cent below average, whilst that for 1959, with a favourable growing season, was almost twice the average. A similar pattern is reflected in the intake data for Newcastle. Data for discharge is shown for home consumption (road and rail) and export (ship). There are discrepancies between the figures for annual intake and discharge, partly through losses by cleaning and partly because some stocks may be held over from one season to the next. No wheat was exported in 1952 and 1957 and only small quantities were shipped in 1958; even in favourable seasons the amount shipped from one month to another varies considerably, with maxima of 55,000 and 75,000 tons from Sydney in 1956 and 1960, respectively, and 40,000 and 50,000 tons from Newcastle in 1956 and 1959.

The last two wheat crops for the State of New South Wales have exceeded 2½ million tons, a figure never before attained, and this Board has been called upon to handle quantities of between 2 and 2½ million tons in the two successive years. These figures compare with the Board's previous record hand-

TABLE I

Throughput (intake and discharge) of wheat in tons at Sydney and Newcastle Terminal Elevators 1951–9 (Jan.–Dec.)

Sydney

Year	Intake	Discharge by			
		Road	Rail	Ship	Total
1951	225,170	94,140	—	168,090	262,230
1952	268,070	126,860	—	—	126,860
1953	330,450	102,150	—	204,260	306,410
1954	273,510	109,030	50,570	129,270	288,870
1955	224,900	49,010	—	128,350	177,360
1956	292,720	103,980	230	204,450	308,660
1957	74,330	117,330	19,160	—	136,490
1958	116,050	93,410	65,750	21,520	180,680
1959	462,150	62,170	5,800	251,820	319,790
Average:	251,930	95,340	15,720	123,080	234,140

Newcastle

Year	Intake	Discharge by			
		Road	Rail	Ship	Total
1951	43,970	18,090	—	16,400	34,490
1952	39,090	27,290	10,000	—	37,290
1953	139,670	15,290	—	95,120	110,410
1954	91,530	10,240	—	88,430	98,670
1955	56,370	4,500	—	16,410	20,910
1956	83,740	8,920	20	74,420	83,360
1957	8,340	15,600	2,540	—	18,140
1958	18,400	12,090	15,010	1,750	28,850
1959	173,250	13,050	480	156,040	169,570
Average:	72,710	13,900	3,120	49,840	66,860

The annual intake of wheat at the Sydney terminal, based on the past two seasons, is now in the vicinity of 1½ million tons; the Newcastle intake is approximately ½ million tons per annum.

ling for one season of approximately 1½ million tons. These two crops have been disposed of by the Wheat Board practically within 12 months of receipt: with local consumption more or less fixed, the whole of the production increase has been either exported or reserved for export and the throughput of the Newcastle and Sydney terminals has increased accordingly. Conditions of the wheat industry in N.S.W. are such that, given favourable weather, production is likely to continue at the present high level and may increase still further.

Weekly fluctuations in intake (Fig. 1) and discharge (Fig. 2) for the Sydney and Newcastle terminals are analysed in detail for 1955–6 and 1959–60. Within periods of high and low intake, weekly fluctuations are comparatively small; during these years maximum weekly intakes at Sydney and Newcastle were 25,000 and 11,500 tons, respectively. Discharge to ships is irregular but variations in home demand (road and rail) are small, the requirements of local mills and manufacturers of breakfast foods remaining reasonably steady from week to week.

Integration of radiation treatment

This outline of the methods used to convey grain during terminal handling, together with the numerical data from Australia, identify the problems likely to arise in integrating the irradiation process at a port. Apart from changes in crop production causing annual variations in the throughput of grain, the location of the irradiation plant within the terminal is of considerable importance. The siting of an irradiation plant at intake or discharge would have to accommodate the following requirements:

1. Very high intake rates from up-country storage (probably 200 to 1000 ton/hr).
2. Peak intake maintained for limited periods only (a few weeks at the beginning of each season's harvest).
3. High discharge rates to boats to reduce dockage to a minimum (probably 500–1000 ton/hr).
4. Infrequent and irregular boat-loading periods (probably totalling not more than 100 days per year).
5. Breaks in intake and discharge (to accommodate hold-ups in truck emptying and the trimming of holds).

These requirements, particularly the high rates of grain handling, clearly make very stringent demands on an irradiation process. On the other hand, internal movements of grain within the terminal – for cleaning, turning, admixing with insecticides, or the filling of fumigation bins – take place at relatively low handling rates of up to 200 ton/hr. The filling of shipping bins in preparation for rapid discharge to boats is also more leisurely. There is little doubt that irradiation can be more readily integrated with terminal handling when the process is buffered from the external influences of intake

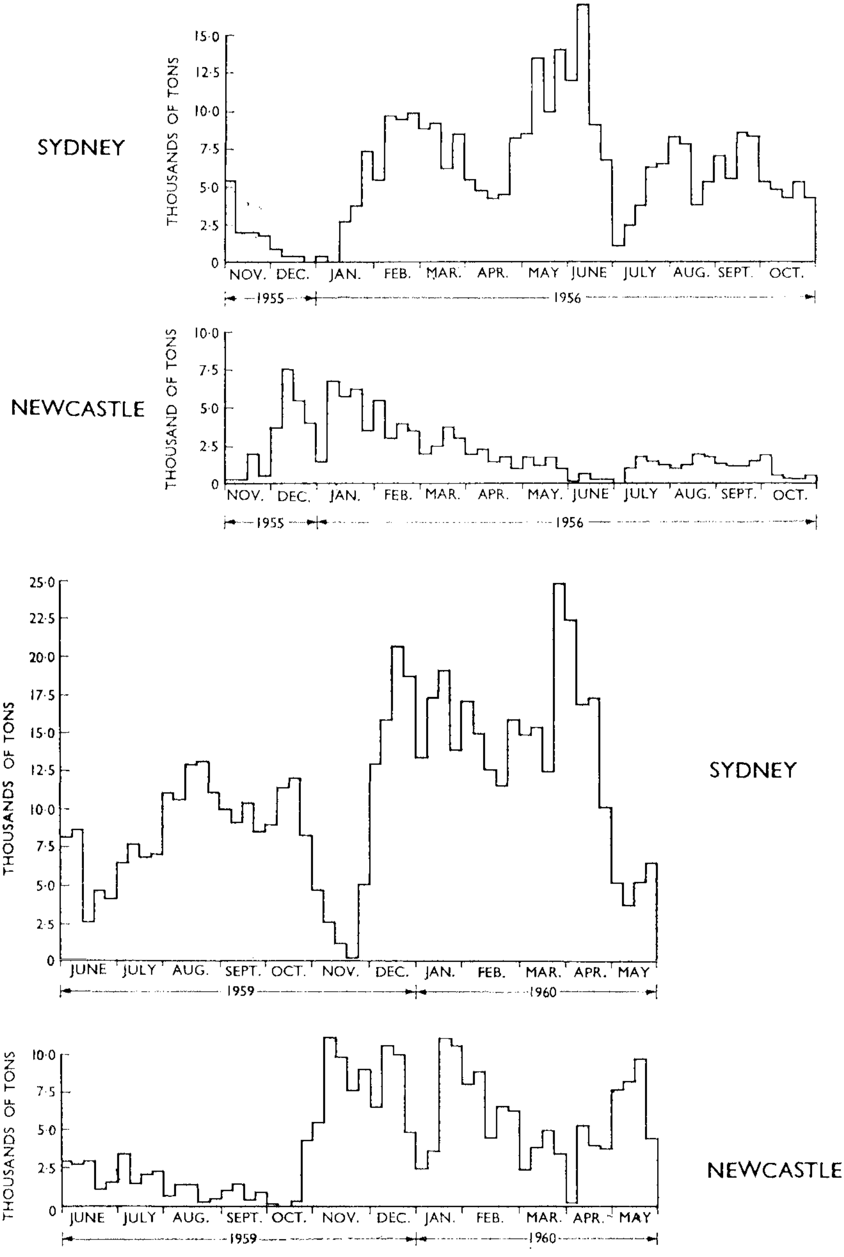


FIG. 1. Weekly intake of wheat at the Sydney and Newcastle terminal elevators, 1955–6 and 1959–60.

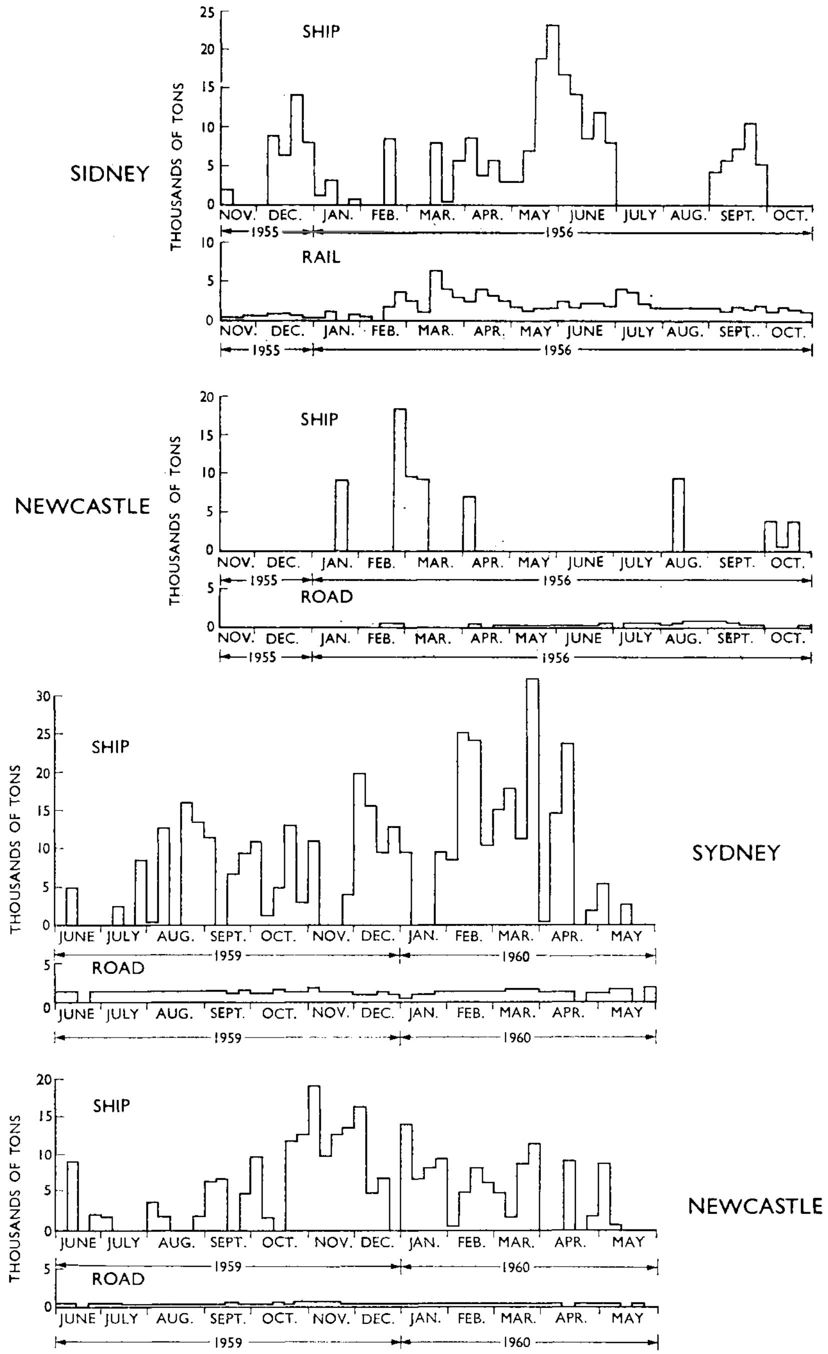


FIG. 2. Weekly discharge of wheat from Sydney and Newcastle terminal elevators, 1955–6 and 1959–60.

and discharge; under these conditions the handling rate of the process can be reduced to a minimum, so maintaining treatment over long periods.

Irrespective of its actual location, the irradiation plant must not impose too severe a restriction on normal handling procedures within the terminal. To be compatible with intermittent demand, conveyor loadings currently in use should be used (at least 200 ton/hr). Plant could, however, be kept in operation for longer periods, perhaps overnight, if fed from a storage reservoir at a lower handling rate (20–40 ton/hr).

The engineering problems of integrating irradiation within existing systems will vary from one grain-handling installation to another, according to the detail of storage and the flexibility of existing conveyors in moving grain from any desired position to another. The functions of import and export terminals are essentially similar – centralised storage and grain handling. Whilst this discussion has examined in some detail the functions of an export terminal, the same pattern is reflected at import; boats are discharged at maximum speed, frequently pneumatically. Movement within the receival storage is more leisurely and the steady discharge inland to road and rail is regulated by transport and demand.

DESIGN PRINCIPLES OF RADIATION DISINFESTATION

Irradiation plant using cobalt-60

Radiation from ^{60}Co is emitted continuously and in all directions such that maximum utilisation of the gamma energy is obtained by surrounding the source with an infinite thickness of grain for the maximum time. The radiation decays at a steady rate, reaching half its intensity in 5·3 years. If a constant supply of grain requiring insecticidal treatment can be provided to surround a source at static bulk density in the form of a solid sphere, most of the radiation would be utilised; the dose of radiation received by the grain would, however, vary considerably unless it were kept in motion, since the intensity of radiation decreases with the square of the distance from the source and there is considerable absorption of radiation by the product. Early suggestions involved introducing into a silo bin, a lattice of sources to provide a uniform dose distribution throughout the grain and to provide chronic irradiation during storage life. This arrangement, however, suffers from many disadvantages; chronic treatment is less effective than acute, a uniform dose rate would be difficult to achieve, thick walls of concrete would be required for adequate shielding, and a separate lattice would be required for each bin. There would also be considerable engineering problems in manoeuvring a lattice of sources to a safe position when the bins are emptied.

Various designs of plant for disinfestation of grain have been given in which the grain is conveyed during treatment; Pertsovskii *et al.*[1] have

designed a semi-industrial installation for treating grain at 0·5 ton/hr with 1850 curies of ^{60}Co. A further design is given by Bibergal *et al.*[2] for treating grain with ^{60}Co at 1·85 ton/hr; the same authors consider the potential of fission products using 3·7 Megacuries to give a throughput of 31 ton/hr. Brownell and colleagues[3] illustrate a gamma irradiation facility using reactor fuel elements to treat flour, meal or grain at 27 ton/hr, and an experimental grain irradiator containing 15,000 curies of ^{60}Co has been suggested to give 10,000 rads at 3 ton/hr.[4] The methods of handling grain for irradiation include screw and pneumatic conveyors, bucket elevators and gravity feed. The screw conveyor suffers from the disadvantage of different rates of flow at the top and bottom of the worm and loss of a proportion of the radiation in the materials of its construction. The density of grain in the air flow of pneumatic conveyors is only about one-sixtieth of static bulk density, and calculations show that with a 10 cm tube (typical diameter of pneumatic plant) the utilisation of gamma radiation would be about 0·5 per cent, 99·5 per cent being wasted outside the operating field. With bucket elevators and the use of a slab type source, the size of bucket may be designed to give a very small difference in dose throughout its volume. The method now in use for the commercial irradiation of packaged commodities[5,6] might be extended, grain being introduced on the outside of the radiation field, and conveyed to an inner circuit close to the radiation source, with the side incident to the radiation reversed when conveying it out. The same principle has been suggested for the treatment of bagged grain in the hold of a ship.[7] The use of buckets or bags, however, is not efficient since a considerable portion of radiation is lost between the units and in absorption by the conveying system.

Fabrications involving gravity feed undoubtedly provide the optimum method for maximum radiation utilisation; the unique flowing properties of grain are exploited to achieve movement at high densities, without absorbing the radiation with unnecessary metal. In an early discussion of this principle Cornwell[8] postulated the use of a single rod source of ^{60}Co, 10 ft long, surrounded by concentric cylinders offering 3 annuli of 28, 56 and 84 ft^3, the grain being fed at different rates, first through the outer annulus and then through the inner ones. In this arrangement the grain could be kept at least 8 in. away from the central source to avoid the high overdosing which is unavoidable without good grain mixing; 210,000 curies would be required to treat grain with 16,000 rads at 25–30 ton/hr with an efficiency of 40 per cent. If a matrix of rods or plates of cobalt-60 is used, self-absorption is reduced and grain can be allowed to flow intimately around the source. Plants of this type can be designed to utilise about 70 per cent of emitted radiation.

The following design utilises 150,000 curies of cobalt-60 to treat 30 ton/hr with 16,000 rads. The radiation source consists of a number of rods of cobalt-60 each about 6 in. long and arranged vertically in a flat plate 4 ft long and 8 in. deep. This array is surrounded by a light metal wall so shaped as to

give a substantially uniform dose around its circumference. This wall is surrounded by another to produce an annulus 4 in. wide. This is surrounded by a third wall with a further gap of 4 in. and again, if desired, by a fourth wall with an annulus of 5 in. All the walls are vertical, and in the arrangement described there are therefore 3 annular flow paths. In use, these are kept filled

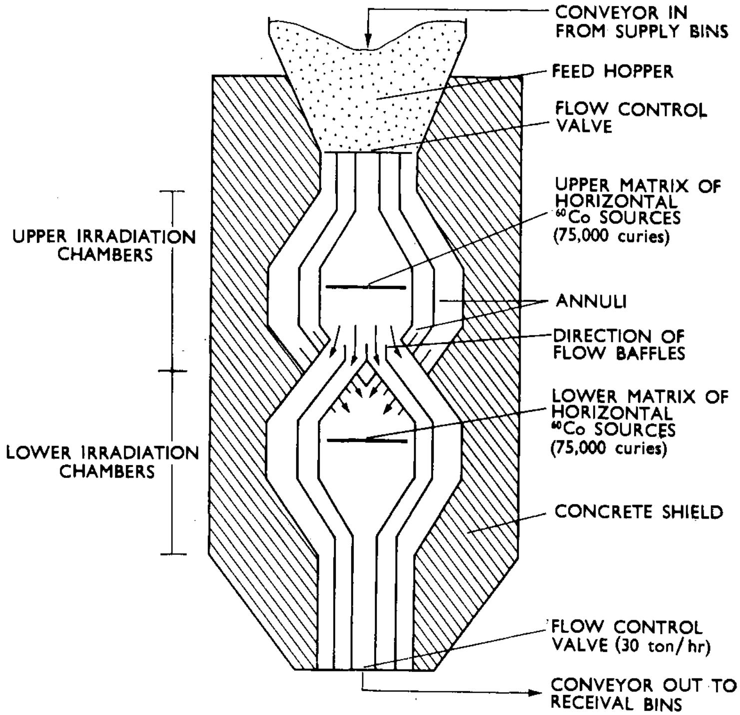


FIG. 3. Diagrammatic section of prototype ^{60}Co irradiation plant for disinfestation of grain.

with grain from a suitably designed hopper, and grain is allowed to flow from the lower end at controlled rates, slower for the outer annulus than for those which it surrounds, so that the residence time of the grain in each path is controlled to give the required dose.

A modification of this design (Fig. 3), affording the same dose and throughput, takes the form of 2 radiation sources in the shape of grids about 2 ft square located one above the other with the rods 2 ft long, parallel and horizontal. In this design, grain flows round the cobalt rods; it passes in a surrounding annulus 4 in. wide and in a further, outer annulus 5 in. wide. Instead of controlling the dose by differential rates of movement, grain leaving the upper chamber (having received a higher dose near to the cobalt rods) is deflected to the annuli of the lower source. Similarly, grain having received

a lower dose in the annuli of the top source, is deflected to the central chamber of the lower source. The distribution of flow rate between the outer paths is controlled by baffles. The grain, after passing both sources, is withdrawn from the bottom of the plant at a substantially uniform rate.

Thus, in the first design the flow rates through the various paths are individually controlled so as to give differing residence time, whereas in the second, differential withdrawal rates are not required. The actual source is also arranged in a slightly different manner in the two systems, in such a way as to affect dose uniformity. The whole is surrounded by a concrete shield of dimensions conforming to the requirements of health and safety – approximately 10 ft × 20 ft in plan and 10 ft × 15 ft high. It is desirable in the design of the prototype to include a safe position to allow withdrawal of the source for purposes of maintenance, engineering research or dosimetry. This is a refinement which gives the prototype greater versatility and allows modifications to be made if required.

All studies on ^{60}Co irradiation plant for disinfestation of grain have so far been theoretical, except for preliminary investigations on the design given above, using a small scale model of a section of one of the annuli. More information is needed on the following:

1. The most effective way of achieving smooth, uninterrupted flow at near static bulk density through probably a dozen valves (or baffles) each at 2–3 ton/hr. A full scale model of a section of the plant may be required to determine the necessary control settings; vibrator systems may be necessary to stop clogging of the grain stream.
2. The most suitable materials for construction to minimise friction and wear, particularly if the design involves grain passing round horizontal sources.
3. The ideal configuration of cobalt rods in relation to the requirements of dose uniformity specified by entomological research.
4. The most reliable conveyor, elevator and valve systems (conventional types) to ensure uninterrupted movement of the grain from the supply bins, and from the plant to receival bins. If the plant runs overnight (16 hr) treating grain at 30 ton/hr and without supervision, it can treat almost 500 tons, or the contents of 2 conventional silo bins (15 ft dia., 60 ft high).

Irradiation plant using accelerated electrons

In the past, electron machines have been criticised as unreliable sources of radiation for industrial processing and the cost of processing has been high. During the last few years, however, considerable advances have been made in the development of insulated core transformers[(9)] and radio-frequency cascade rectifiers[(10)]; direct current power outputs of the electron beam are available up to 30 kW and at energies up to 3 MeV. In the present state of

technology these two machines provide the cheapest form of electron processing. To achieve good uniformity of irradiation a machine of 20 kW beam power at 3 MeV is necessary to treat grain with 16,000 rads at 200 ton/hr using 50 per cent of the incident radiation. Apart from costs, a major disadvantage of electron energies above 3 MeV is the production of a low neutron flux which can produce small amounts of radio-activity in the processed

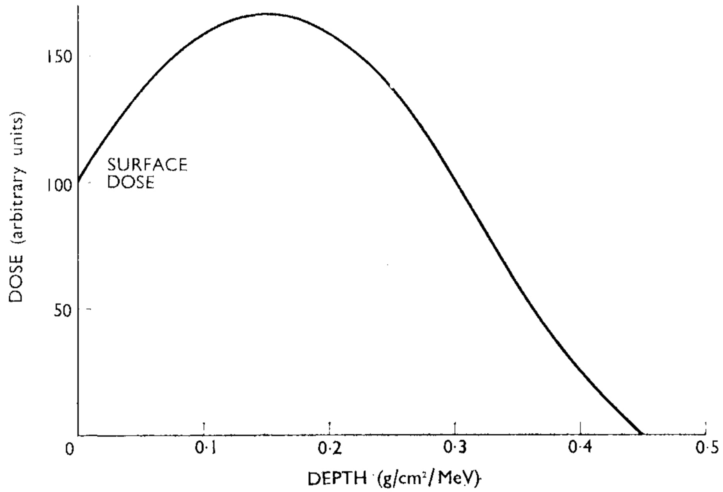


FIG. 4. Distribution of energy (dose) in the depth of material irradiated with accelerated electrons.

material. Although this activity may be extremely difficult to detect and short-lived, it could raise special problems in the clearance of the foodstuff for human consumption by the appropriate Health Authorities. Discussion on the use of accelerated electrons is therefore limited here to consideration of the two types of machine mentioned above.

Penetration of electrons of 3 MeV or less is small; grain must therefore be treated in a thin uniform layer, and if large throughputs are required, either the grain velocity must be high or the layer very broad. As with isotope plant, not all the radiation can be absorbed, particularly if a low ratio of maximum to minimum dose is required. Taking a typical commercial wheat, Manitoba No.2, individual grains have a mass of between 0·03 and 0·035 g, equivalent to a mass per unit area of 0·25 g/cm^2. The useful range of 1 MeV electrons is 0·3 to 0·35 g/cm^2. The dose received, however, is not uniform since there is a build-up of energy within the irradiated material (Fig.4); if an arbitrary value of 100 is assigned to the dose at the surface, the dose rises to 167 at 0·15 g/cm^2, falling to 100 at 0·3 g/cm^2 and to 50 at 0·36 g/cm^2. Irradiation from both sides simultaneously allows the thickness which receives a reasonably uniform dose to be increased to 0·7 g/cm^2 on account of the additive

dose at the centre. It follows that, in order to achieve a good measure of dose uniformity with 1 MeV electrons, the grain stream must be limited to not more than three grains thick. To achieve throughputs of 200 ton/hr with so thin a grain stream requires high velocities, since the maximum available width of the scanning window is at present 75 cm. Experiments with a gravity fall system at Wantage have shown that when grain is dropped from 6 m at 200 ton/hr down a tapered duct, it emerges from an aperture 75 cm × 2 cm at a velocity of about 11 m/sec at a density of 0·67 g/cm^2. Although this thickness is theoretically within the capabilities of a 1 MeV machine, irradiating from two sides, examination of high speed film shows some non-uniformity of the grain stream. Such a stream has places where it is more than three grains thick, which would result in underdosing of some grains, and less than three grains thick resulting in loss of efficiency where the stream is too thin to absorb all the incident radiation. If, however, 3 MeV electrons are used, the useful thickness of the grain stream can be increased to 2·5 g/cm^2, enabling improved uniformity of dose deposition, higher grain handling rates or lower grain velocities.

In the following design originating from the Wantage Research Laboratory the grain is contained in a large hopper and falls through an aperture which controls the rate of flow (an aperture of 75 cm × 8·5 cm allows a flow of 200 ton/hr). The hopper removes irregularities in conveyor loading and should be large enough not to overflow. The grain falls into a vertical duct 45 cm high, the duct being tapered towards its base to an aperture of 75 cm × 5 cm. Grain emerging through this aperture falls between opposed electron beams (3 MeV) at about 3 m/sec and at a density of 0·5 g/cm^3. The electron beams are arranged to point downwards at a small angle from the horizontal; this avoids use of special windows to resist impact by grain and subsequent wear, and also the mutual irradiation of the scanners should the grain stream stop. If the electron beam has a spot diameter of 7·5 cm and a scan frequency of 500/sec, then each grain will be treated 10 times at a dose rate of 6·4 Mrad/sec, the total treatment time for each grain being $\frac{1}{400}$ sec. It is not necessary for the machine to cut out if there is a failure in grain supply, but an electronic system is required if the machine fails so that the untreated grain is passed back to the feed hopper. It should be possible with the prototype to vary the thickness of the grain stream at irradiation to accommodate cereals of different density and to allow such modifications in dose uniformity as are indicated by entomological research. The machine and ducting is surrounded by an adequate shield, of similar dimensions to that suggested for ^{60}Co plant, but allowing for variations in duct height.

Some supporting engineering research on the machine prototype is desirable. It would be an advantage to build a full-scale model of the prototype (without the electron machine) to test wear on materials. The reliability of the plant, particularly the machine, requires evaluation after installation. The

model should be made to accommodate changes in throughput by altering the effective height of fall and thus the velocity at exit, so retaining the efficient use of 3 MeV electrons. This might be achieved by incorporation of baffles in the duct.

DEVELOPMENT OF PILOT PLANT

There is already sufficient evidence from the biological studies to justify initiation of engineering development of plant for radiation disinfestation of grain. Whilst it is unlikely that engineering problems will be a limiting factor in the application of the irradiation process, the development of pilot plant is necessary to provide first-hand experience of the yet unforeseen problems of industrial application, both physical and biological, and to give opportunity for obtaining an economic evaluation of the process under practical conditions. The development of pilot plant is also necessary to provide the presently required stimulus for commercial appreciation of radiation disinfestation as an alternative insect control procedure.

Development can of course take place in countries other than the one in which pilot plant is to be installed for test. The most suitable country for installation is one which affords irradiation the maximum advantage: a grain exporting country, where storage in the hinterland precludes effective measures of control or protection before grain reaches the port, or possibly a country in the tropics importing infested grain for long-term storage. It should be situated in a country where there is a need to treat a high proportion of the grain passing through the elevator, and where there are at least minimum facilities for entomologists and engineers concerned with the elevation of the pilot plant in operation. Tropical and sub-tropical areas are especially suitable for such a pilot plant as these, indeed, provide conditions in which effective insect control procedures are most desirable. There would not at present appear to be an immediate demand for such an installation in the U.K., although it could be installed in a country exporting grain to this country. Ideally it is required to test both ^{60}Co and electron machine plants in the same location, enabling a practical comparison with circulatory fumigation. This ideal could most readily be achieved by installing the plant in a grain elevator projected for construction, using the expertise of industrial grain-handling organisations to evaluate the most effective method for integrating the plant for most effective operation.

COSTS OF CONVENTIONAL INSECT CONTROL METHODS COMPARED WITH IRRADIATION TREATMENT

Costs of chemical treatment

In making a comparison of costs between irradiation and chemical methods it cannot be too greatly emphasised that the cost of processing grain by irradiation is closely related to annual throughput. The practice with chemical methods is often to treat only grain known to be contaminated and the cost per ton is not usually affected by total quantity treated annually. The cost of treating a particular consignment will nevertheless be lower per ton for large consignments than for smaller ones.

Very little information is available on the practical field costs of chemical control of insects in stored foodstuffs. Apart from the cost of the insecticide or fumigant, inclusions must be made for handling, deterioration of machinery during application, transport of staff and equipment, labour and supervision. Commercial servicing companies must also allow for their greater overhead charges and for profit. The chemical treatment bearing most resemblance in results to irradiation is fumigation. Assuming that large quantities of bulk grain in silos are being treated under skilled supervision as the responsibility of a Government Department or a Government-sponsored Grain Marketing Board, the cost of fumigation with methyl bromide falls between 1*s.* 0*d.* and 2*s.* 0*d.*/ton. When similarly large quantities of grain in floor storage, in bulk or in bags, are fumigated under gas-proof sheets, the costs per ton range from 1*s.* 6*d.* to 2*s.* 6*d.* (Parkin, personal communication).

The price of methyl bromide itself is about 6*d.* per treated ton (Knight, personal communication). The cost of treating grain by circulatory fumigation in a bulk bin installation is estimated by Turtle (Table II) to range from 8*d.* to 1*s.* 8*d.* The actual cost varies with the size of the installation and labour rates in different parts of the world. With fumigation in barges or lighters there is again quite a wide variation in costs depending on the type of craft used, the tonnage involved and the distance service crews have to travel; working on a closed craft a cost of 1*s.* 6*d.* to 2*s.* 0*d.*/ton might be expected, or on an open craft, requiring sheeting, 3*s.* 0*d.* to 3*s.* 6*d*/ton. The cost of fumigating stacks under gas-proof sheets averages 3*s.* 6*d.* to 4*s.* 0*d*/ton (Knight, personal communication). Turtle (Table II) gives figures for 1953 of 1*s.* 3*d.* to 2*s.* 6*d.*/ton on lighters and 4*s.* 3*d.*/ton for fumigation under sheets. The pricing for different methods of fumigation is influenced largely by supply and demand. As a guide for other methods of fumigation, Knight in discussion estimates the cost of treating bulk grain in the U.K., with aluminium phosphide, at 2*s.* 0*d.* to 3*s.* 0*d.*/ton; in extensive treatments at low rates of application of 3–4 tablets per ton in Australia, the average of a number of estimates in 1960 was 1*s.* 6*d.*/ton. Knight estimates fumigation with mixtures of ethy-

lene dichloride and carbon tetrachloride, or treatment with ethylene dibromide, or cyanogas, at figures ranging from 5*s*.0*d*. to 8*s*.0*d*./ton. These are considerably higher than the costs given by Turtle (Table II) for 1951.

The cheapest form of treatment with contact insecticides (Parkin, personal communication) is by applying a dust or spray to a grain stream during mechanical handling. In this way a high degree of protection can be achieved for

TABLE II

Cost of fumigation and insecticide treatments

Treatment	Commodity	Cost/ton
*MeBr. fumigation in lighters	88 treatments involving 108,600 tons of cereals, cocoa, rice in London, 1953	1/3*d*–2/6*d* neglecting hire of lighters
*MeBr. fumigation of bags under sheets	21 treatments involving 25,250 tons of cereals in U.K.	4/3*d* including cost of covering stacks and depreciation of sheets
†MeBr. fumigation of bagged grain under sheets	Range of conditions in Australia, 1960	1/4*d*–2/9*d*
*MeBr. in circulatory fumigation plant	Bulk bin installations	Estimate: 8*d*–1/8*d* depending on type of installation
*Calcium cyanide fumigation	16,800 tons of grain in silos in U.K., 1951	2/4*d* including turning cost
*Carbon tetrachloride mixture	14,700 tons of grain in silos in U.K., 1951	9*d* (no turning cost)
*Phostoxin tablets (Aluminium phosphide)	Grain in silos used for trials in U.K., 1957	2/6*d* including 1/– for turning
†Phostoxin tablets	Grain in upcountry and terminal elevators in Australia	Average: 1/6*d*

* Turtle, E.E. *Intern. J. appl. radiation and isotopes*, **6**, 222 (1959).
† Cost figures provided by grain handling authorities in Australia.

up to 12 months at a cost of approximately 1*s*.6*d*. to 4*s*. 0*d*./ton. Knight has suggested figures of 1*s*. 6*d*. to 2*s*.0*d*./ton for insecticidal spraying and dusting of bagged cereals, or empty bins with, for example, malathion or lindane. Turtle *(loc. cit.)* gives the cost of 3*s*. 0*d*. for treating bulk grain with pyrethrum dust in the U.S.A., or 4*s*. 6*d*. if the cost of turning is included.

There are clearly a variety of factors which influence the cost of chemical treatment, and it is almost impossible to give a figure which is accurate. So much depends on the conditions under which the grain is to be treated, and the figures given must be accepted only as a guide. The costs suggested by Parkin, for instance, apply to large quantities of grain treated under the supervision of a well-organised, non-profit-making body, and the actual costs may often be in excess of the ranges given. For the purposes of comparing chemical treatment with irradiation it would be advisable to use a lower limit of 1*s.*0*d.* and an upper limit of 2*s.*0*d.* since the cost of circulatory fumigation, with which irradiation is most comparable, falls within this range.

Cost of treatment with cobalt-60

The cost of radiation processing using ^{60}Co is most favourable with treatment spread throughout the year, over 24 hr/day. A cost analysis is presented in Table III based on a plant efficiency of 70 per cent. It is assumed for the purposes of calculation that the plant is located at a port terminal handling an average of two large grain cargoes per month, each of 12,000 tons. The throughput for irradiation is therefore 288,000 ton/annum. The market price for ^{60}Co for sources of this size is 5*s.*/curie. To accommodate a handling rate of 200 ton/hr a source of 1 million curies is required, and the capital cost for plant is about £$\frac{1}{4}$ million. Variations in cost for different annual throughputs (Fig. 5) range from 9*s.*0*d.*/ton for 150,000 tons to 3*s.*0*d.* for 500,000 tons. Clearly at his handling rate the capital cost is high and operating costs are not competitive with conventional methods; capital cost could, however, be reduced by lowering the handling rate and spreading treatment over a longer period. In the analysis for 70 ton/hr (Table III) the throughput (288,000 tons) is maintained continuously 24 hr/day for half the year. The running of conveyors outside normal working hours is not, however, in accord with present handling practice, and to allow treatment to continue overnight it may be necessary to feed the plant from an existing grain storage bin or to construct special reservoirs, a feed bin filled during the day and a receiver bin emptied the following day. With present-day costs for extensions to storage at 6*s.*0*d.*/bushel, constructional costs would be near £40,000. There would also be an extra handling cost, estimated at 4*d.*/ton. The lowest capital costs for gamma irradiation plant are obtained with treatment spread over the full year at the lowest possible handling rate. With treatment of 288,000 tons at 35 ton/hr two reservoir bins would be required each of 850 tons; the capital cost for irradiation plant drops to £50,000 and processing costs range from 4*s.*6*d.* to just under 2*s.*0*d.* for 50,000 and 300,000 tons, respectively. The latter figure is competitive with costs for conventional measures of insect control. Continuous treatment at 35 tons/hr would necessitate reorganisation of present grain-handling procedures, since movement of grain is most often confined to working hours. Elevator managers might be reluctant to adopt

a process not readily integrated with existing handling operations. If the cost of cobalt-60 were to fall to 1 *s.* 0*d.* per curie, treatment costs would fall to one-third of present estimates; a marked drop in the price of cobalt seems unlikely.

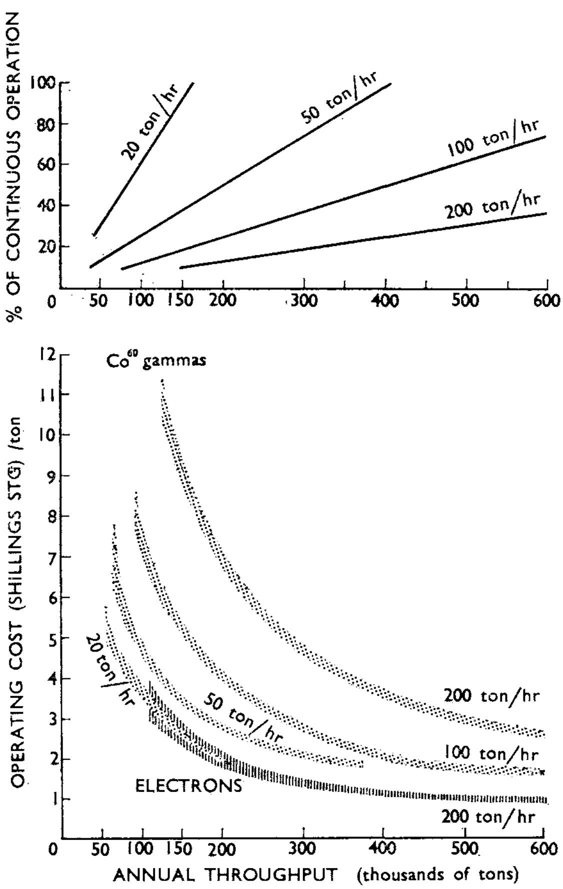


FIG. 5. Estimated cost of disinfesting grain by irradiation with cobalt-60 gammas and electrons in relation to handling rate and annual throughput.

Costs of treatment with accelerated electrons

Using an average of manufacturers' costs for the electron machine, an analysis of costing for radiation disinfestation of grain at 200 ton/hr is given in Table IV. Less shielding is required than for ^{60}Co plant of equivalent rate, the height of the shield being influenced by the magnitude of the free fall of grain. The machine itself also contributes to a lower capital cost, so that the outlay on plant for treating with electrons at 200 ton/hr is similar to that

for isotope plant operating at one-third of this rate. The operating costs at low throughput, 50,000 to 200,000 ton/annum, are very similar to treatment with ^{60}Co at 20 ton/hr (Fig. 5). At annual throughputs above 200,000 tons

TABLE III

Disinfestation of grain by gamma irradiation (^{60}Co). Analysis of costing

Handling rate (ton/hr)	200		70		35	
Throughput (ton/yr)	288,000		288,000		288,000	
Operation (hr/yr)	1440		4115		8230	
Source strength (curies)	1,000,000		350,000		155,000	
Item	Capital Investment £	Operating Cost £	Capital Investment £	Operating Cost £	Capital Investment £	Operating Cost £
^{60}Cobalt at 5*s.*/curie*	250,000		87,500		38,750	
15% replacement		37,500		13,125		5813
5% interest, 5% amortization		25,000		8750		3875
Plant (source fabrication, shield and grain flow system)	30,000		20,000		12,500	
5% interest, 10% amortization		4500		3000		1875
Labour and overheads at £2/hr		2880		8230		16,460
Total	280,000	69,880	107,500	33,105	51,250	28,023
Operating cost/ton.		4*s.*10*d.*		2*s.*4*d.*		1*s.*11*d.*
Extra handling cost/ton		Nil		4*d*		4*d*
Additional capital cost for feed and receival storage	Nil		40,000		20,000	

* ^{60}Co cost is quoted at U.K. market price, encapsuled for insertion. The Canadian price is very similar and the U.S. is slightly lower (60 cents/curie). The price is an artificial one depending on method of accounting. Future changes in market price cannot be anticipated; it depends very much on continued production and use of ^{60}Co.

the cost of disinfestation falls below 2*s.*0*d.*/ton, to about 1*s.*0*d.*/ton at 500,000 tons. Clearly the cost of disinfesting grain with accelerated electrons is highly competitive with the present-day costs of fumigation.

TABLE IV

Disinfestation of grain by electron irradiation. Analysis of costing

Handling Rate:					200 ton/hr
Capital Investment:	Electron machine				£ 75,000
	Plant (shield and grain flow system)				£ 20,000
	Total				£ 95,000
Operating Costs:	5% Interest, 10% Amortization /yr				£ 14,250
	Labour and overheads/hr				£ 2
	Utilities and replacement for accelerator/hr				£ 2
Cost in relation to operating hours and throughput:					
Operating hr/yr:	500	1000	1500	2000	3000
Tons treated/yr:	100,000	200,000	300,000	400,000	600,000
Interest and Amortization /hr:	£ 28·5	14·3	9·5	7·2	4·8
Labour, overheads and replacement/hr:	4	4	4	4	4
Total/hr:	£ 32·5	18·3	13·5	11·2	8·8
Operating cost/ton:	3*s*. 3*d*.	1*s*. 10*d*.	1*s*. 4*d*.	1*s*. 2*d*.	11*d*.

WHOLESOMENESS AND ACCEPTABILITY OF IRRADIATED GRAIN

It is a pre-requisite of radiation processing that the dose level worked out for disinfestation must produce no adverse effects on the quality of the grain, or on its acceptability to the consumer; there must be no reduction in its nutritional adequacy, or production of toxic compounds. Before health clearance may be given long-term animal feeding studies are therefore essential. A variety of animals have been used to examine the nutritive value and possible toxicity of irradiated grain; these include rats, mice, dogs, poultry, guinea-pigs and some insects. Entomological research has shown that 16,000 rads can be accepted as a reliable dose for radiation disinfestation and in certain circumstances it may be more than adequate.

Nevertheless, many tests of wholesomeness have been made at doses considerably higher, particularly in the U.S., where treatments with 2·79 Mrad and 5·58 Mrad have been used in a programme concerned with food sterilisation and the elimination of bacteria.

Toxicity

Details of the most pertinent studies on the feeding of animals with irradiated grain and cereal products are summarised in Table V. Tests of acute toxicity to study food consumption and growth were conducted over periods of 8–12 weeks. Chronic toxicity has been assessed over several generations using the parameters of food consumption, growth, longevity, reproduction and tumour incidence. Histopathological tests have been made at the end of short and long feeding periods, and tests for carcinogenicity have usually been made with mice after feeding for 80 weeks. In the United States, feeding tests have also been extended to human subjects, but only for restricted periods.

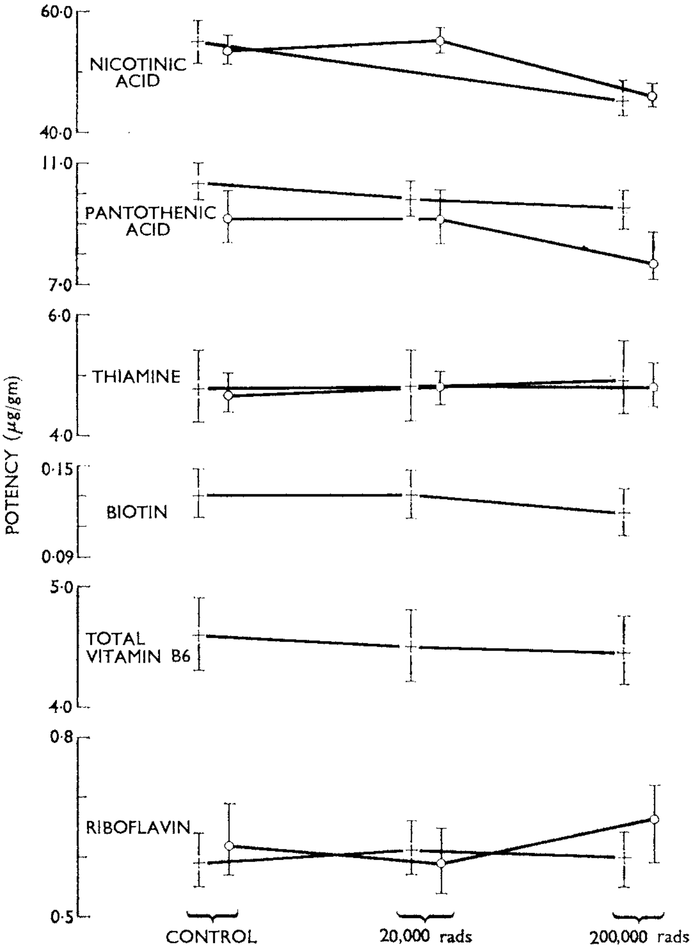


FIG. 6. The effect of gamma radiation on some B-complex vitamins in Manitoba wheat. Mean and standard errors (Kennedy, T.S. *J. Sci. Food Agr.* (in press).).

TABLE V

*Summary of animal feeding data on irradiated cereals**

Test Subject	Test Material	Irradiation Dose Level	No. of Animals	Duration of Test	Measurements made	Comments	Reference
Rat	Bread	2·79 Mrad and 5·58 Mrad	—	8 weeks	Growth, Food Consumption, Histopathology	Satisfactory	11
Rat	Whole corn Bread Military Cereal Bar	2·79 Mrad and 5·58 Mrad	10/gp.	8–12 weeks	Growth, Food Consumption, Pathology	No gross toxic symptoms	12
Rat	Bread	25,000 rad and 50,000 rad	40/gp.	12 weeks	Growth, Blood Enzymes	No indication of toxicity	13
Rat	Macaroni Pound cake Crackers	2·79 Mrad and 5·58 Mrad	10/gp.	8 weeks	Growth, Food Consumption	Satisfactory	14
Rat	Corn Starch (incorporated with other irradiated items)	3·72 Mrad	31/gp.	2 years (4 gen.)	Growth, Reproduction, Histopathology	Wholesome apart from some vitamin destruction	15
Rat	White flour	37,000 rad and 74,000 rad	50/gp.	2–3 years (4 gen.)	Growth, Breeding, Longevity, Histopathology	Satisfactory	16, 17

* I am grateful to my colleague, Mr. F. J. Ley of the Wantage Research Laboratory for the compilation of this Table.

TABLE V *(continued)*

Test Subject	Test Material	Irradiation Dose Level	No. of Animals	Duration of Test	Measurements made	Comments	Reference
Rat	Military Cereal Bar	6 Mrad	54/gp.	2 years (4 gen.)	Growth, Reproduction, Metabolisable Energy, Tissue Enzymes	Growth normal except 4th gen. F. Other indices normal except cytochrome oxidase in males. No tumours	18
Rat	Wheat	20,000 rad 200,000 rad	60/gp.	2–3 years (4 gen.)	Growth, Reproduction, Longevity, Histopathology	No indication of toxicity	19, 20
Rat	Wheat	10,000 rad	—	2–3 years (4 gen.)	Reproduction, Histopathology		21
Rat	Wheat	10,000 rad	—	3 gen.	Blood Data, Growth, Fertility		22
Mouse	White flour	37,000 rad and 74,000 rad	200/gp. (2 strains)	80 weeks	Tumour data, Growth, Mortality		23
Mouse	Wheat	10,000 rad	—	5 gen.	Weight, Fertility, Blood data, Tumour data		22
Mouse	Wheat	20,000 rad 200,000 rad	120/gp.	80 weeks	Food Consumption, Growth, Tumour data		19

TABLE V *(continued)*

Test Subject	Test Material	Irradiation Dose Level	No. of Animals	Duration of Test	Measurements made	Comments	Reference
Dog	Wheat flour	37,000 rad and 74,000 rad	4/gp.	24 weeks	Growth, Food Intake, Haematology	No difference	24
Dog	Wheat flour	37,000 rad and 74,000 rad	4/gp.	104 weeks	Reproduction, Blood data, Histopathology	No difference	25
Poultry	Corn, Oats, Wheat, Barley	2·79 Mrad	60–90/gp.	13 months	Body Weight, Food utilisation, Egg production, Egg fertility, Chick data, Histopathology	No evidence of toxicity	26
Poultry	Mixed Cereals	19,500 rad	50/gp.	6 months	Weight, Egg production, Pathology, Biochemical tests	Performance of animals unchanged	27
Guinea-pigs	Commercial diet of mixed cereals	3 Mrad	18	2 months	Growth, Reproduction, Lactation	Normal	28
Guinea-pigs	Semi-synthetic diet: oats, corn starch, corn oil	2 Mrad	—	—	—	Diet used for rearing germ-free animals. No abnormalities	

TABLE V *(continued)*

Test Subject	Test Material	Irradiation Dose Level	No. of Animals	Duration of Test	Measurements made	Comments	Reference
Insects Insects	Wheat Wheat	50,000 rad 1 Mrad	No deleterious effect of irradiated diet on reproductive potential of flour beetle, grain weevel, grain moth. No effect on development of grain weevils				29, 30
Humans	Various cereal products	2·79 Mrad	9 or 10 subjects	15 days	Clinical laboratory tests: haematology, organ function, urine analysis	No toxic effects during or after tests	31

Nutritive value

Carbohydrates are unaffected nutritionally even at high doses of radiation. The biological value of maize protein and wheat gluten is unaffected by treatment with 2·8 Mrad but the digestibility of maize protein is lowered by 5 per cent at 9·3 Mrad.[32] Experiments with rats carried out in support of the entomological studies at Wantage confirm that the biological value of wheat protein is unchanged up to 1 Mrad.

Radiation has little effect on the B-complex vitamins in Manitoba wheat (Fig. 6). Microbiological assay could detect no change at 20,000 rads and only slight losses in nicotinic and pantothenic acids at 200,000 rads. Resistance of B vitamins to radiation damage in wheat and other cereals has also been found by other workers[33–38]; Russian data, however, indicates that thiamine is sensitive in white flour with a 10 per cent loss reported at 50,000 rads and 65 per cent loss at 150,000 rads.[39] On the other hand, chemical assays of thiamine in Pearl barley and Buckwheat also carried out in the U.S.S.R., indicate an increase in this vitamin at 50,000 rads. Fat-soluble vitamin E is normally regarded as radiosensitive, but chemical assays at Wantage have shown little destruction at 20,000 rads and 200,000 rads.

Legislation

In the Federal Republic of Germany, ionising radiations for the treatment of foodstuffs is forbidden. With this exception, the current legislative regulations of the majority of countries make no specific reference to irradiation processing of foods; since they prohibit any treatment which renders food injurious to health, control of new processes is regarded as adequate. At a recent international meeting in Brussels, in which all aspects of the wholesomeness of irradiated foods were reviewed, it was concluded that "while it is premature to give health clearance for the irradiation of food in general, sufficient evidence has been accumulated on the treatment of certain food items to allow their appraisal by appropriate authorities". In France, draft regulations for the radiation processing of foods have already been prepared. In the U.S.A., submission to the Food and Drug Administration for the health clearance of wheat and wheat products, treated with gamma radiation from sources with maximum energy not exceeding 2·2 MeV and to provide an absorbed dose from 20,000 to 50,000 rads was approved in August 1963. A similar submission, incorporating full experimental data is shortly to be made in the U.K.; there is now sufficient evidence to support the conclusion that treatment of cereals with the low doses required for disinfestation does not give rise to toxic or carcinogenic compounds and that nutritional damage is negligible. These findings are also supported by data obtained and still being accumulated on other foods treated at very much higher doses.

Acceptability

A number of studies have been made of the effects of irradiation on hard[40–43] and soft wheats,[34,44] flours,[45–52] cereal products[53–57] and wheat gluten[58,59] with respect to physical properties,[60,61] induced biochemical changes and the behaviour of doughs. Studies have also been made on the cooking[57] and brewing properties of rice.[62,63] Most of these studies have been carried out at very high doses, adverse effects being consistently observed at doses of 1 Mrad and above, but few studies have been carried out at dose levels of economic interest for insect control. The studies of Milner and Yen,[64,65] for example, are concerned with the effects on breadmaking of doses required for the destruction of microflora responsible for the heating and biochemical deterioration of grain stored at high moisture contents, while some of the European investigators have been concerned with the possible improvement of flours by irradiation.[48,66]

Whilst biochemical changes of academic interest have been recorded, the results of many investigations have not been assessed in relation to the milling and manufacturing properties of the cereals. A number of papers refer to organoleptic appraisals to evaluate the threshold for off-flavours and off-odours,[44,67] usually with inadequate numbers of judgements, with the result that some of the conclusions are based on insufficient information and cannot withstand rigorous statistical treatment.

Extensive tests (unpublished) have been carried out at Wantage, in collaboration with four U.K. cereal laboratories, into the effects of gamma radiation on English wheat treated at 20,000, 125,000, 500,000 and 1,000,000 rads. It was held in bulk storage for periods up to 1 year and milled to 60 per cent, 70 per cent and 80 per cent extraction rates. The results for the two lower doses are presented in Table VI; no changes were obtained at 20,000 rads with the exception of a slight darkening of the flour. Treatment at 125,000 rads increased the water absorption of the flour to an extent comparable with a change in moisture content of 0·5 per cent. The same dose caused a measurable decrease in viscosity, but only a very slight increase in maltose value. Only three of the four cereal laboratories were able to show statistically perceptible changes in dough properties at 125,000 rads, and these were a little more marked at the higher levels of extraction. With the exception of modifications in viscosity, irradiation at 125,000 and 500,000 rads produced only minor changes in the grain, comparable with relatively small changes in extraction rate. In most cases, alterations in the physical properties of the dough by irradiation at doses up to 500,000 rads were less severe than can be obtained with heat conditioning, the use of flour improvers, or which result from 6–12 months' storage. It is clear that doses many times higher than that proposed for the disinfestation of grain may be used before causing changes of commercial significance, with the reservation that they might be sufficient to influence the trade against wheat which had been treated, if given

TABLE VI

Physical, chemical and baking properties of flour milled to 60, 70 and 80% extraction from irradiated English wheat. Means of determinations made at 3-monthly intervals during storage for 1 year. Values underlined differ significantly from the control at 1% Probability (Cornwell, unpublished).

Test	Dose (× 10³ rads)									Number of determin-ations
	60% extraction			70% extraction			80% extraction			
	Control	20	125	Control	20	125	Control	20	125	
Moisture content (%)	14·6	14·8	14·6	14·4	14·5	14·5	14·5	14·6	14·6	10
Water absorption (gall./sack)	13·8	13·8	$\underline{14\cdot2}$	14·1	14·2	14·3	14·4	14·3	14·5	10
Colour grade	5·0	$\underline{5\cdot3}$	$\underline{5\cdot4}$	5·4	$\underline{5\cdot7}$	$\underline{5\cdot8}$	7·1	7·1	$\underline{7\cdot3}$	15
Amylograph max. viscosity (B.U.)	864	898	$\underline{745}$	937	888	$\underline{705}$	821	826	$\underline{688}$	5
Hagberg Number	11·6	12·2	12·8	11·8	12·0	13·0	12·0	12·4	13·8	5
Maltose value	1·01	1·02	1·06	1·04	1·06	1·08	1·18	1·16	1·18	5
Gas production	6·0	5·9	6·0	6·1	6·1	6·2	6·4	6·5	6·7	5
(1) Extensibility (E)	15·5	14·4	15·7	15·5	14·7	16·3	15·2	16·3	15·6	5
(1) Resistance (R)	314	372	366	364	404	378	348	324	364	5
(1) $\frac{E \times 10}{R}$	0·54	0·41	0·47	0·46	0·38	0·45	0·46	0·52	0·45	5
(2) Extensibility (E)	19·1	18·9	18·6	19·9	19·7	19·7	20·3	19·9	$\underline{18\cdot9}$	5
(2) Resistance (R)	58·6	65·8	61·0	58·0	58·2	62·8	52·0	55·2	57·8	5
(2) $\frac{E \times 10}{R}$	3·5	3·2	3·2	4·0	3·7	3·3	4·6	4·0	3·6	5

TABLE VI (continued)

Test	Dose (× 10^3 rads) 60% extraction Control	60% extraction 20	60% extraction 125	70% extraction Control	70% extraction 20	70% extraction 125	80% extraction Control	80% extraction 20	80% extraction 125	Number of determin-ations
(3) Stability	53	52	57	56	58	63	58	58	64	5
(3) Strength	31	31	35	34	36	37	36	34	37	5
(4) Extensibility	7·7	7·3	7·0	7·1	7·0	6·9	7·1	6·9	6·4	5
(4) Stability	6·2	6·5	8·5	6·8	6·6	7·3	4·8	6·1	6·8	5
(4) Resiliency	6·1	6·1	6·5	5·9	6·3	6·6	5·9	5·9	6·4	5

(1) Determinations by H. Simon Ltd., with Simon Research Equipment. } using different scales
(2) Determinations by the British Baking Industries Research Association with Simon Research Equipment. } using different scales
(3) Determinations by Drs. Kent-Jones & Amos with the Chopin Alveograph.
(4) Determinations by Aynsome Laboratories with non-instrumental methods on personally evolved scales.

TABLE VII

Taste panel results of the effects of gamma radiation on the organoleptic properties of biscuits and bread made from irradiated soft English wheat. Means of determinations made at 3-monthly intervals during storage for 1 year after irradiation. Values underlined differ significantly from the control at 1% Probability (Cornwell, unpublished).

BISCUITS

Paired comparison tests
Preference intensity (%) for irradiated sample

Extraction rate %	Dose comparison (rads × 10^3) 0–20	0–125	0–500	0–1000
60	99·4	99·2	96·5	92·7
70	101·1	99·3	97·8	93·2
80	99·2	99·3	98·8	93·2

Means of 240 tests per treatment.

BREAD

Paired comparison tests
Preference intensity (%) for irradiated sample

Extraction rate %	Dose comparison (rads × 10^3) 0–20	0–125	0–500	0–1000
60	101·3	101·2	98·1	96·3
70	102·0	99·4	97·2	96·7
80	99·5	98·5	98·6	98·5

Means of 120 tests per treatment.

BISCUITS

Triangle test
Percentage of correct observations

Extraction rate %	Dose comparison (rads × 10^3) 0–20	0–125	0–500	0–1000
60	39·5	43·1	43·9	49·5
70	36·9	40·5	40·6	52·6
80	38·7	39·0	41·1	41·7

Means of 375 tests per treatment.

BREAD

Triangle test
Percentage of correct observations

Extraction rate %	Dose comparison (rads × 10^3) 0–20	0–125	0–500	0–1000
60	38·1	45·5	31·1	50·5
70	37·7	36·7	44·1	54·8
80	41·2	45·7	41·8	47·3

Means of 175 tests per treatment.

Note. A mean of 100 per cent is obtained in the paired comparison test if the preference intensity is equal for control and irradiated samples. A mean of 33·3 per cent is obtained in the triangle test if odd samples are selected correctly by chance.

a choice. The slight toughening of the gluten may be desirable when irradiated soft wheats are admixed with imported varieties.

In organoleptic studies carried out at Wantage with irradiated wheat, 11,000 assessments were made with a panel of selected members, using the paired comparison test and triangle test to detect off-odours and flavours in bread and biscuits (Table VII). Only with biscuits and using the triangle test, was it possible to detect the presence of irradiated wheat treated with 20,000 rads. Using the paired comparison test, a reduction in preference intensity was not apparent until the dose level was raised to 500,000 rads. The presence of irradiated wheat was more readily detected when milled to a low extraction rate.

One further aspect of the acceptability of irradiated cereals which remains to be considered is the effect of treatment on germination. It is customary in most countries to retain locally on farms that grain which is intended for seed; it does not usually enter the same channels as the bulk of cereals destined for storage or marketing and is therefore most unlikely to experience treatment by irradiation. Nevertheless, a number of studies on irradiated cereals have included a test on germination[41,64,65] and there is a considerable body of literature devoted to the effects of ionising radiations on the production of chromosomal aberrations and the consequent effect upon inheritance.[68,69] Soderholm and Walker,[70] conclude that doses of 10,000 to 200,000 rep do not prevent the germination of wheat treated with high energy electrons, but doses of 40,000 to 200,000 rep, not only markedly reduce the number of embryos reaching the surface of the soil, but completely check their continued growth. The possible effects of radiation on barley intended for malting are therefore of significance. Work on the biochemical effects of irradiation on a medium grade malting barley[71] has shown that germination is only seriously reduced at 500,000 rads, although shoot production is impaired by 8000 rads and plumules completely stunted at and above 50,000 rads, In addition, germination is uneven at low dose levels. Since a uniform germination is essential for the production of good quality malt, this factor might well prejudice the use of irradiation for disinfestation where the level of infestation is slight.

THE FINDINGS OF ENTOMOLOGICAL RESEARCH

The results of the entomological studies have already been discussed in the individual papers. They are now summarised, highlighting those aspects which remain for further investigation.

Radiation dose

The severity of radiation damage to reproductive and somatic tissues varies with dose. Complete and immediate kill of insects is obtained at 300,000 to

500,000 rads,[53,72,73] complete kill a few days after irradiation at about 100,000 rads, and complete reproductive sterilisation followed by death a few weeks after irradiation at 10,000 to 20,000 rads. On economic grounds, radiation disinfestation of grain should make use of a sterilising dose to inhibit reproduction and cause death some days later.

The minimum effective dose for sterilising pupae and adults of the grain weevil *(S.granarius)* is 16,000 rads, death occurring 2–3 weeks after treatment. All eggs and larvae are prevented from maturing, and thus from reproducing, by 5000 rads. When infested grains containing all developmental stages are irradiated with 16,000 rads, only those containing late pupae are able to produce adults, these being sterile. Any measure of overdosing above that required for effective treatment constitutes wasted energy and a consequent increase in operating costs. Irradiation treatment cannot ensure that all grains receive the minimum effective dose without some measure of overdosing. It is therefore pertinent to ask what degree of control can be achieved with treatment of a high proportion of grains at the required dose and the remainder at sub-sterilising doses.

Sub-sterilising doses of ionising radiation cause deleterious effects in the gametes, which keep weevil populations suppressed to a low level for many months; using 10,000 and 12,000 rads, populations of *S.granarius* rose to only 6 per cent and 0·2 per cent respectively, after 7 months. With small populations, doses of 10,000 and 12,000 rads can give complete control and if processing follows shortly after irradiation these doses are also adequate for suppressing populations of up to 1 million adult weevils. The cost of irradiation is thus appreciably reduced.

Evidence with the grain weevil has also indicated that the reproductive potential of insects treated at 6000 rads (i.e. 40 per cent of the minimum effective dose) is further reduced by one-third when outnumbered by sterile insects at a ratio of 5:1.

Sterilised adults can also provide partial protection to grain against reinfestation when the number of sterile insects is large compared with the numbers of insects reinfesting the commodity. This protection is afforded by the insemination of fertile females with sterilised sperm whilst the irradiated population remains alive. Sterile sperm remains competitive with fertile sperm within the female for at least 4 months. If irradiation is carried out so as to allow a proportion of sterile insects to remain alive for a longer period, the reproductive potential of contaminants can be further reduced. The "biological" protection afforded by irradiation is a distinct advantage over fumigation, particularly where storage conditions in warm climates are physically inadequate to combat reinfestation. More work is necessary, however, with the grain weevil and other species to explore the optimum conditions for exploiting this useful property.

Susceptibility of species

Thirteen cereal pests tested for susceptibility to irradiation showed a high level of sterility at 6000 rads.[74] Of the 5 species of beetles most frequently encountered in wheat and barley (*Table VIII) 4 are completely sterilised by 16,000 rads, and fertility of the fifth, *O. surinamensis* is reduced to 0·1 per cent. Grain and rice weevils are killed (100 per cent) by 16,000 rads, but 2 per cent of adult *R. dominica*, 4 per cent of *O. surinamensis* and 10 per cent of adult *T. castaneum* survive. Grain and rice weevils are among species most *rapidly* killed by irradiation. Moth species are more resistant than beetles to sterilisation and killing by irradiation.

TABLE VIII

Susceptibility of insect species to 16,000 rads

Species	% sterility	% kill
**Sitophilus zeamais*	100	100
**Sitophilus granarius*	100	100
Tribolium confusum	100	100
**Rhizopertha dominica*	100	98
**Tribolium castaneum*	100	90
**Oryzaephilus surinamensis*	99·9	96
Lasioderma serricorne	99·9	—
Plodia interpunctella	99·7	no reduction in longevity
Cadra cautella	96	
Anagasta kühniella	93	

Strains from infested storage vary considerably in susceptibility to killing by irradiation; 16,000 rads has proved effective against 30 wild strains of *S. granarius* from various parts of the world; strains of weevil and flour beetle collected from Australian storage are also controlled by this dose. There is no evidence at present to suggest that strains of insects resistant to insecticides are more tolerant of radiation.[75]

The chemical composition of cereal media influences the rate of development of *T. confusum*; rapidly developing beetles are killed more rapidly by irradiation. The rate of development of *S. granarius* on different whole cereals could not be correlated with chemical composition, and is probably affected by other factors. The type of cereal supporting the infestation is unlikely to influence the efficacy of irradiation.

Dose rate

Chronic irradiation is less effective than acute: adult *S. granarius*, *T. confusum* and *O. surinamensis* are more readily killed by dose rates at the higher end of the range, 1500–4700 rads/hr. The fertility of adult *O. surinamensis* is

also similarly modified. At very high dose rates, radiation sterilisation of pupae of *S. granarius* is in small measure reduced by treatment with accelerated electrons (mean dose rate $8{\cdot}6 \times 10^6$ rads/hr) compared with ^{60}Co gamma radiation (75,000 rads/hr). In studies from which results must be treated with reserve, high dose rate electrons were also inferior to gamma radiation in killing and sterilising adult *S. granarius*. It is postulated that the optimal dose rate for radiation disinfestation of grain lies between 10^4 and 10^6 rads/hr: this suggestion requires confirmation since it may have a significant influence on the choice of plant and handling rate for an industrial process. American studies[(53)] report no difference in the insecticidal efficiency of electrons, gammas and X-rays.

Temperature

High temperatures before irradiation (30 °C) sensitise grain weevils to killing by irradiation. High temperatures (30 °C) during irradiation increase resistance to killing. High temperatures after irradiation accelerate the rate of mortality only. Temperature does not modify the susceptibility of the grain weevil to sterilisation. Responses to temperature, dose fractionation and dose rate are closely associated with the ability of the insect's tissues to recover from radiation damage. The recovery of reproductive integrity of *S. granarius* is slow; nevertheless it should not be assumed that variations in temperature before, during or after irradiation could not modify the sterilising effects of irradiation on other species, with higher metabolic rates, or different rates of gamete maturation. Temperatures experienced in grain storage practice are unlikely to influence the efficacy of irradiation for the control of grain weevils, but more work should be carried out with other species. Low temperatures after irradiation may retard rate of death, but feeding activity is also much reduced. Cornwell[(76)] has shown that grain weevils treated with 16,000 rads ingest approximately half the amount of food compared with untreated adults.

Temperatures above 26 °C, induced by metabolic heat of larval populations, increase the resistance of adult *S. granarius* to killing by irradiation, but again have no effect on susceptibility to sterilisation. The rearing conditions examined in laboratory experiments were equivalent to the worst conditions likely to be encountered in commercial practice where "hot spots" of infestation occur as a result of prolonged storage. The lowest level of infestation tested was roughly equivalent to that at which compulsory treatment is necessary in the United States. No loss is envisaged in the efficacy of the process at insect densities normally encountered in commercial storage.

Oxygen tension

A low oxygen tension at irradiation, sufficient to anaesthetise adult grain weevils in 30 min, increases their resistance to killing and sterilisation by irradiation. Since this condition is unlikely to be encountered in grain storage practice, particularly with movement of the grain during irradiation, the efficacy of the process is not likely to be jeopardised.

Dose fractionation

Fractionated treatments of radiation allow the repair of tissues to radiation damage and increase survival; 16,000 rads, may however, be given to *S. granarius* in at least 4 fractions over a maximum period of 5 days without modifying susceptibility to killing. The level of sterility in adults afforded by fractionated treatment with intervals of up to 1 day is also equal to that with continuous doses. Low doses of radiation for the control of eggs in packaged cereal products cannot be fractionated without detriment to the process.

Population size

With an infestation rate of 0·1 per cent (1 adult/1000 grains) a typical terminal silo bin, holding 200 tons of grain, (the capacity of a bin) contains 5 million adults.* A population of 10 million *S. granarius*, in all stages of development, was effectively sterilised and killed by 16,000 rads in experimental bulk storage. Considerable confidence can therefore be attached to the use of 16,000 rads for radiation disinfestation of grain for an industrial process. Populations ranging from one thousand to one million adults treated with 14,500 rads showed some redevelopment of infestation 5 to 8 months after irradiation.

SUGGESTIONS FOR FURTHER RESEARCH INTO ENTOMOLOGICAL ASPECTS OF RADIATION DISINFESTATION

It may be some years before radiation disinfestation is accepted by industry as an alternative to insect control by toxic chemicals. A number of reasons for this delay have already been discussed; they include health clearance, problems of integrating the process into grain handling procedures, terminal design and not least the economics of the process. There will consequently be some delay before entomological problems, yet unforeseen, become ap-

* An infestation rate of 3 insects/1000 grains is unacceptable for milling.

parent in the use of irradiation plant for industrial treatment of grain. There are, however, a number of entomological problems upon which research should now be initiated.

Efficacy of irradiation in the treatment of heating grain

The minimum effective sterilising dose for disinfestation (16,000 rads) results in death of insects some time after treatment. The longevity of larval stages within the grain has not been examined in detail. Fumigation of heating grain can reduce the temperature quickly through death of immature stages within 24 hr. Treatment by irradiation may be less effective; with species other than weevils there is evidence that irradiation causes reduced metabolic activity (lethargy) during the post-irradiation survival period; feeding activity is much reduced with adults before death. Temperature and respiration studies are required with immature stages of grain and rice weevils to examine post-irradiation effects on metabolism.

Susceptibility to radiation of insects to diapause

The period required for a visible expression of radiation damage after treatment (e.g. death) is closely associated with rate of metabolism and cell division. The likely application of radiation disinfestation to the treatment of wheat in warm climates, particularly in Asia, suggests the need to examine the radiation susceptibility of insects subject to diapause and of *Trogoderma granarium* in particular.

Susceptibility of insects resistant to chemical treatments

No tests have yet been carried out to assess whether strains of stored product insects resistant to insecticides are in any way modified in susceptibility to irradiation; with development of the process at port terminals, some chemical treatment may be carried out prior to irradiation at export or at import. Irradiation may play a valuable role in the future, should a marked increase occur in the resistance of insects to insecticides.

If sub-sterilising doses of radiation are used for short-term protection of commodities, mutagenic changes in insects may possibly accelerate the development of resistance to chemicals; Japanese workers have confirmed the existence of a genetic factor, using ionising radiations, in studies of the mode of development of resistance to chemicals.

Possible induction of resistance to radiation

Only limited studies (unpublished) have so far been carried out to examine the possible induction of insect resistance to radiation, and these only with the grain weevil. If resistance develops through a single major gene it may become

apparent in the F_2 generation; if through a particular combination of minor genes, then screening to the F_4 or F_5 generation should be adequate.

Attempts to induce resistance to radiation in bacteria *(E. coli)* has resulted in only a small increase in resistance, of about two-fold. Furthermore, no evidence has been found to support the hypothesis that resistance to radiation is under genetic control and situated at a particular locus. It seems probable, therefore, that insects which have a far slower rate of multiplication would not develop resistance to radiation. A number of major grain pests should, however, be tested at different sterilising pressures to ascertain the likelihood of this factor becoming a disadvantage to the irradiation process.

Sensitisation of insects to radiation damage

The cost of treating grain by irradiation is already competitive with fumigation. Any method of sensitising insects to irradiation (e.g. processes involving pre- or post-heating) may further reduce operating costs. No studies have been carried out with pests of stored products on the interaction of heat (above 30 °C) and irradiation; studies with adult grain weevils have shown that high temperatures (up to 30 °C) sensitise insects to subsequent irradiation, and result in more rapid mortality when applied after irradiation.

Differentiation between sterile and fertile insects

Port Inspectorate are responsible for classifying imports on the degree of infestation. As sterile insects survive for a short time after irradiation, there may be some need to distinguish between live fertile and sterile insects. A rapid evaluating technique is thus required; sperm mobility may be a useful indicator, and in certain insects, atrophy of the gonads may be apparent to superficial examination. Biochemical tests or behavioural responses should be examined.

Reproductive potential of sub-sterilised insects

Studies with *Oryzaephilus surinamensis* have shown that irradiation of adults with 16,000 rads reduces fertility of the F_1 and F_2 generations to 35 per cent. Studies are required with other species to determine the extent of reduced fertility and the number of generations over which reduced fertility is maintained.

Dose rate

With only a few exceptions, radiation susceptibility studies with insects of stored products have been carried out with low dose rates from ^{60}Co. A few have involved electron dose rates of up to 10 Mrad/hr, but none have in-

volved treatment at near 10 Mrad/sec, which will be experienced in the radiation treatment of grain with machines at 200 ton/hr. All have involved pulsed electrons, no studies having been carried out with the recent (direct current) machines.

Present evidence suggests that chronic irradiation is less effective than acute because of biological repair processes to radiation damage; on the other hand, oxygen may be depleted at very high dose rates, more rapidly than it is replaced, so that tissues are treated under intra-cellular anoxia. Treatment under depleted oxygen is known to increase the resistance of grain weevils to killing and sterilisation by irradiation. A study of dose rate effects from chronic to acute levels is necessary to establish the thresholds for repair and oxygen effects. In common with similar studies which have been made with chemicals (concentration × time studies) temperature should be included as an additional variable. Non-pulsed electrons should be tested.

THE NEED FOR ACTION

This account of the entomological work carried out in the U.K., and the review of studies into wholesomeness and acceptability of irradiated grain, has demonstrated the technical feasibility of ionising radiations for the control of insects infesting stored cereals. There are still a number of gaps in our knowledge, but the evidence reported here is sufficient to justify engineering studies and an appraisal of the irradiation process under practical conditions.

The control of insects in stored foodstuffs is an international problem and merits collaborative effort for its solution. This is particularly so with the application of a process requiring international agreement on the clearance of irradiated foodstuffs for human consumption. It is gratifying that the International Atomic Energy Agency in conjunction with the United Nations Food and Agriculture Organisation is actively seeking a location and funds for the initiation of pilot plant studies.

Attention is often drawn to the over-production of cereals in certain parts of the world and the present lack, or inefficient use of proven chemical methods of insect control. Radiation disinfestation is an additional control procedure which merits a place in supplementing existing insecticidal techniques. It is never likely to replace present practices, but may be considered as an alternative where conditions are favourable for its application.

Technically and economically, radiation disinfestation is a potential competitor with fumigation. Radiation processing is compatible with bulk grain handling in large modern facilities. During the next 5 to 10 years an increasing number of bulk storage facilities with automated mechanical handling will be projected for construction in tropical areas, and an increasing fraction of the world's yield of harvested cereals will pass in trade between the

"over producers" and those areas of the world which make an increasing demand for food. With the demand for higher standards of food hygiene, public opinion is likely to decry the increasing contamination of foodstuffs with harmful insecticidal residues. With new storage installations currently being built it is of vital importance that if the process of irradiation offers any practical advantages over modern fumigation plant, the opportunity for its installation should be seized quickly.

DECEMBER 1963

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